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Comparison of thermally pasteurized and ultrasonically pasteurized blueberry juice (Vaccinium corymbosum) and an investigation of blueberry juice effect on lipid oxidation during microencapsulation of poly-unsaturated fish oils

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COMPARISON OF THERMALLY PASTEURIZED AND ULTRASONICALLY PASTEURIZED BLUEBERRY JUICE (*VACCINIUM CORYMBOSUM*) AND AN INVESTIGATION OF BLUEBERRY JUICE EFFECT ON LIPID OXIDATION DURING MICROENCAPSULATION OF POLY-UNSATURATED FISH OILS

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ABSTRACT

Blueberries have received considerable attention due to their content of health promoting compounds such as polyphenols and anthocyanins. Thermal pasteurization (TP) is known to cause degradation of anthocyanins and juice color. Microencapsulation, particularly with incorporation of blueberry antioxidants, can improve the stability of menhaden (MO) and salmon (SO) fish oils rich in omega-3 fatty acids. The objectives of this research were to study the effect of TP and continuous ultrasonication processing on the microbiological, chemical and physical properties of blueberry juice (BJ) and to determine the effect of BJ on MO and SO lipid oxidation during microencapsulation.

Fresh blueberries were blanched, blended and then centrifuged to obtain the juice. BJ samples were pasteurized at 80, 85 and at 90°C for 1, 2, 3, 4 and for 5 min. Unpasteurized BJ was pumped at 24 mL/min and at 93.5 mL/min to the continuous flow cell of an ultrasonic processor, where it was sonicated at three treatment levels (40, 80 and 100% amplitude). Sonicated (non-TP) and unsonicated (TP) juices were analyzed for coliforms, total aerobes, yeasts and molds. Total anthocyanin content, total phenol content, antioxidant activity, ◦Brix, titratable acidity, pH and color were also determined for both sonicated and unsonicated juices. Emulsions prepared with each type of fish oil having 0, 5 and 10% BJ were separately spray dried and microencapsulated powders were produced. All the powders were evaluated for microencapsulation efficiency (ME), color, peroxide value, anisidine value, and fatty acid methyl ester profile.

Sonication at higher intensities reduced microbial counts equivalent to TP. Sonication did not affect BJ color or anthocyanins content while TP showed reduction of anthocyanins and change in juice color with increased temperature and time. Furthermore TP juices showed signs of Maillard browning with increased temperature and time. All microencapsulated MO and SO powders had high ME with low surface oil content. All

powders containing 10% BJ showed less lipid oxidation during emulsification and spray drying than powders containing 5% BJ and 0% BJ. This study demonstrated that ultrasonication can be an alternative pasteurization treatment to TP and that BJ reduced lipid oxidation in fish oils during microencapsulation.

CHAPTER 1 LITERATURE REVIEW

1.1 Ultra-sonication

Sonic waves having frequencies (>20 kHz) above the range audible to humans are defined as ultrasound (Soria, and Villamiel 2010; Elmehdi and others 2003). Ultrasound range can be divided into three different frequency ranges: diagnostic ultrasound (1-10 MHz), high frequency ultrasound having 100 KHz- 1 MHz with low sound intensity (0.1-1 W/cm^2) and low frequency power ultrasound in the kHz range (20-100 kHz) with high sound intensity (10-1000 W/cm^2) (Ashokkumar and Kentish 2011). High frequency ultrasound applications include food quality analysis, non-destructive inspection and medical imaging. Power ultrasound which has high energy is widely used for many commercial applications such as emulsification, homogenization, extraction, low temperature pasteurization, degassing, defoaming, particle size reduction and viscosity alteration (Lorimer and others 1996).

1.1.1 Power Ultrasound

Power ultrasound employs a more powerful form of ultrasound at a lower frequency (20-100 kHz). It can provide the mechanical effect of cavitation in liquid systems which can alter physical and chemical properties of food depending on the type of material involved. When ultrasound waves pass through a medium they will induce a series of compression and rarefaction waves on the molecules of the medium. Initially this will enforce a sinusoidal acoustic pressure (P_a) in addition to the hydrostatic pressure acting on the medium (Soria and Villamiel 2010). This acoustic pressure can be represented by the following Eq. 1.1 using wave frequency (f), time (t) and the maximum pressure amplitude of the wave ($P_{a,max}$). $P_{a,max}$ is directly proportional to the power input of the transducer (Bates and Patist 2008).

$$P_a = P_{a,\,max} \sin(2\pi ft) \qquad (1.1)$$

At lower intensities (amplitude) ultrasonic waves with low acoustic pressure induce motions within the liquid which cause a mixing effect known as acoustic streaming. Once ultrasound intensity (amplitude) is sufficiently high, the rarefaction cycle exceeds the attractive forces of the liquid molecules resulting in formation of cavitation bubbles in the liquid medium. These bubbles are formed from the gas nuclei within the fluid and are distributed throughout the liquid. After a period of few cycles, the bubbles will grow into a critical size which makes them unstable and violently collapse. The collapsing bubbles will create energy accumulated hot spots which can generate high temperature (5000 K) and pressure (1000 atm) resulting in high shear energy zones and turbulence in the cavitation zone of the liquid (Soria and Villamiel 2010). This phenomenon can be easily understood by looking at Fig. 1.1, which shows the changes in the bubble during the wave cycle of ultrasonication. The extent of cavitation is affected by energy (WL^{-1}), intensity (Wcm^{-2}), medium viscosity, surface tension, vapor pressure, nature and concentration of dissolved gas, temperature and pressure of the treatment and presence of solid particles (Soria and Villamiel 2010).

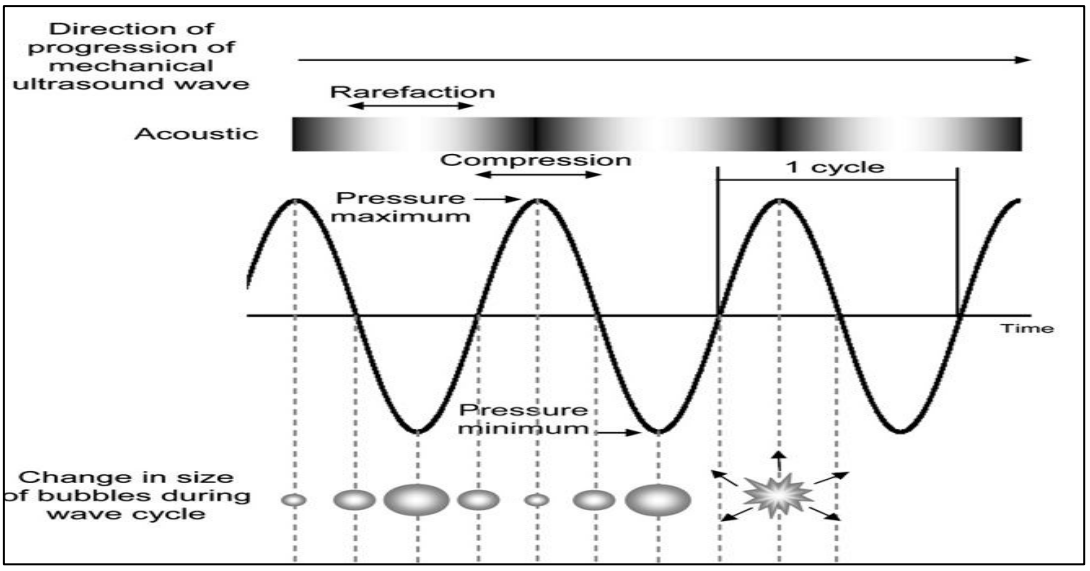


Figure 1.1 Ultrasonic cavitation (Source: Soria and Villamiel 2010)

1.1.2 Ultrasonic Liquid Processor

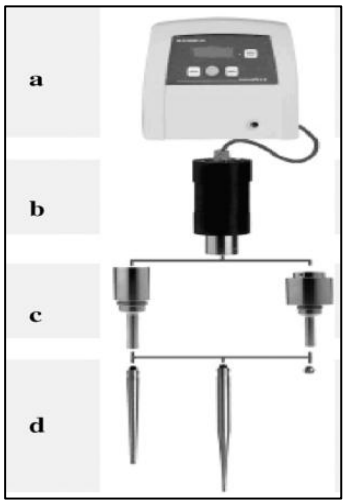


Figure 1.2 Ultrasonic probe (a) generator: (b) Ultrasonic converter: (c) standard and booster horns: (d) Probes. (Source: Capelo-Mart*R*nez and others 2009)

A typical ultrasonic processor (Figure 1.2) consists of a generator which transforms main voltage into high frequency electrical energy at 20 kHz. This is fed to an ultrasonic converter element called a piezoelectric transducer which transforms supplied electrical energy to 20 kHz mechanical vibratory energy (Povey and Mason 1998). The standard and booster horns provide the ability to change the sonication amplitude. The probe located at the bottom of the equipment, is used to transmit ultrasonic vibration energy into the solution to produce intense cavitation (Capelo-Martrnez and others 2009).

1.1.3 Ultrasonic Processing Parameters

- Energy

Ultrasonic energy is expressed as energy input per volume of treated material (khL^{-1}). Energy input is the power output (W) and the flow rate (L/h) of the liquid through the ultrasonic processor which relates to the time of exposure (Bates and Patist 2008). Figure 1.3

shows the general relationship between ultrasound energy and flow rate for several ultrasonic applications.

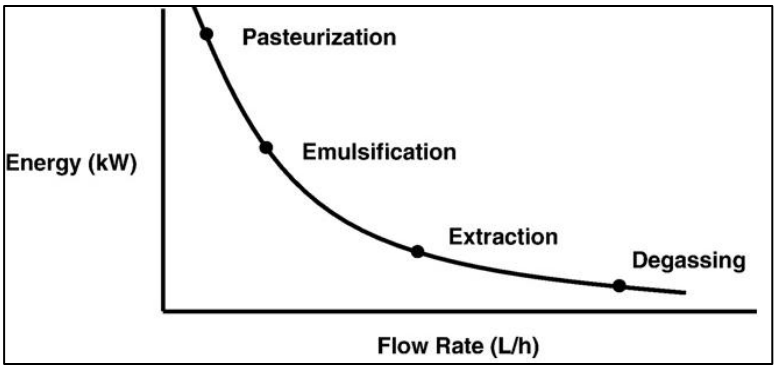


Figure 1.3 Graph of flow rate (L/h) and energy (kW) for ultrasound applications (Source: Bates and Patist 2008)

- Frequency

The formation of cavitation bubbles within a liquid is more difficult with increasing frequency from KHz to MHz. According to previous research, frequency is inversely proportional to the bubble size, thus power ultrasound generates larger bubbles in the cavitation zone resulting in higher temperatures and pressures. Increasing frequency requires an increase in the intensity of the applied sound in order to produce cavitation. High frequencies form short cycles of compression and rarefaction ultrasonic waves which cannot separate liquid molecules to form voids, and hence produce no cavitation. Therefore most industrial ultrasound applications utilize the frequency range between 16-100 kHz in order to obtain the cavitation effect (Capelo-Martrnez and others 2009).

- Intensity

Intensity refers to the power output per surface area of the sonotrode (W/cm^2). Intensity of sonication is porportional to the amplitude of ultrasonic vibration, which results in an increase in the sonochemical effect with increasing amplitude (Bates and Patist 2008).

Although there is a minimum intensity required to achieve cavitation, higher amplitudes are not always necessary to obtain the desired sonication effect. Also employing higher amplitudes may cause rapid deterioration of the ultrasonic transducers which provide only liquid agitation without any cavitation effect. But increased amplitude may be essential for high viscosity samples which need more mechanical vibration in order to promote cavitation (Capelo-Mart*R*nez and others 2009).

- Solvent

Ultrasonication applications are commonly performed in water, but depending on the requirement, less polar liquids can also be used. However, solvent selection should consider the liquid properites of the solvent since high viscosity and high surface tension of the solvent tends to retard the cavitation effect (Capelo-Mart*R*nez and others 2009).

- Temperature

Although an increase in temperature leads to an increase the number of cavitation bubbles, the collpase of these bubbles is hindered by the increased vapor pressure. Thus cavitation is better attained at lower temperatures. Contrarily, the temperatrue has an effect on liquid vapor pressure, surface tension and viscosity where an increase in temperature reduces the viscosity allowing more violent cavitation bubbles. A compromise between temperature and cavitation should be achieved in order to optimize processing (Muthukumaran and others 2006).

- External Pressure

The number of cavitation bubbles is reduced by the increased external pressure due to increase of the cavitation threshold (Muthukumaran and others 2006). Conversely the increased pressure in the liquid will increase pressure in the collapsed bubbles resulting in a

more rapid and violent collapse providing a greater sonochemical effect (Lorimer and Mason 1987). The ultrasonication process can be intensified by increasing the external pressure without the need of increasing the amplitude (Bates and Patist 2008; Hielscher 2005).

- Bubbled Gas

Dissolved gas in a liquid can favor the ultrasonication process by acting as nuclei for caviation. In order to increase the cavitation effect, the gas must be bubbled continuously into the solvent. The monoatomic gases like He, Ar and Ne can be used for this purpose (Capelo-Mart*R*nez and others 2009).

1.1.4 Inactivation of Microorganisms

The food industry is considering ultrasonication as a potential non thermal preservation technique due the previous research findings related to its effect on microbial inactivation. Many researchers believe that the inactivation is mainly due to direct cavitation damage to microbial cell membranes (McKellar and others 2003). But some research findings showed that the inactivation can be achieved without the effect of cavitation. The ultrasound assisted microbial inactivation shows different sensitivities based on size, shape and species of the microorganisms (Barbosa-canovas and others 2011). According to previous research data, bigger cells show more inactivation than smaller ones, and coccal forms are more sensitive than rod shaped bacteria. Also gram positive are more resistant than gram negative bacteria and aerobes show more sensitivity than anaerobic bacteria. Furthermore physiological condition of the cells also effect the rate of inactivation with younger cells being more sensitive than older cells, and spore forms show higher resistance than vegetative cells. Based on previous research findings, ultrasound has shown its potential ability to destroy food borne pathogens such as *E coli*, Salmonellae, Ascaris, Giargia, Cryptosporidium

cysts and polio virus. The effect is increased when applied with other anti-microbial methods such as heat and pressure (Ashokkumar and Kentish 2011).

1.1.5 Modes of Ultra-sonication Processing

Power ultrasound can process a liquid in three different modes.

- Sonication

The microbial inactivation caused by sonication at sub lethal temperatures shows a lower killing rate compared to other modes. However ultrasound alone has the ability to destroy bacterial cell walls. In many cases higher intensities are required for complete sterilization (Feng and others 2008).

- Thermo Sonication

Thermosonication refers to the combined application of sonication with mild heat. In 1987, a research group found the synergistic effect of ultrasound and temperature where increased temperature increases the ultrasound effectiveness (Feng and others 2009). According to previous research data, sonication combined with heat has a greater lethality than temperature only treatment. The researchers demonstrated that the lethality is mainly due to the extensive physical damage on the bacterial cell envelope in the form of wrinkles, ruptures and perforations. However, there is a maximum temperature that exists for each organism where beyond this point sonication does not show increased inactivation (Feng and others 2008).

- Mano Sonication

Sonication treatment combined with moderate pressure, termed as mano-sonication, is the most effective sonication form of microbial inactivation. Increased lethality under

moderate pressure was suggested to be the effect of increased intensity of cavitation. In 1999, researchers discovered that the low frequency ultrasound inactivation of *Listeria monocytogenes* increased when the applied pressure increased from ambient to 200 kPa (McKellar and others 2003). An increased pressure beyond the moderate level does not provide an increased rate of inactivation. It should be noted that the pressure level employed in mano-sonication is not in the lethal range as in high pressure processing (Feng and others 2008).

- Mano Thermo-sonication (MTS)

Sonication treatment combined with mild heat and pressure is known as mano thermo-sonication has exhibited enhanced microbial inactivation in many research applications (Feng and others 2009).

1.1.6 Application of Power Ultrasound in Food Processing

Power ultrasonication has many applications in the food industry for its mechanical, chemical and bio-chemical effects. Mechanical uses include crystallization of fats and sugars, degassing, destruction of foams, extraction of flavorings, filtration and drying, freezing, mixing and homogenization and tenderization of meat. The bio-chemical and chemical applications mainly involve bactericidal action, effluent treatment, modification of growth of living cells, alteration of enzyme activity and sterilization of equipment (Ashokkumar and Kentish 2011; Feng and others 2008).

1.2 Blueberries

Blueberry is a common name for the group of flowering plants belonging to the genus *Vaccinium*, section *Cyanococcus*. The high bush, low bush and rabbit eye are the types of blueberries grown in North America. The high bush blueberry, also called cultivated blueberry is developed from wild high bush blueberry species which are grown in 35 states

and two provinces in Canada (Girard and others 2006). These berries are larger in size and sold as both fresh and frozen.

Table 1.1 Blueberry composition (Source: USDA 2004)

Nutrients		Blueberries (100 g)
	Food Energy (Kcal)	57.00
Proximate	Protein (g)	0.74
	Total Lipid (fat) g	0.33
	Carbohydrate (g)	14.49
	Dietary Fiber (g)	2.40
	Ash (g)	0.24
	Water (g)	84.21
Minerals	Calcium (mg)	6.00
	Copper (mg)	0.06
	Iron (mg)	0.28
	Magnesium (mg)	6.00
	Manganese (mg)	0.34
	Phosphorous (mg)	12.00
	Potassium (mg)	77.00
	Selenium (mg)	0.10
	Sodium (mg)	1.00
Vitamins	Vitamin C (mg)	7.70
	Thiamin (mg)	0.04
	Riboflavin (mg)	0.04
	Niacin (mg)	0.42
	Pantothenic Acid (mg)	0.12
	Vitamin B-6 (mg)	0.05

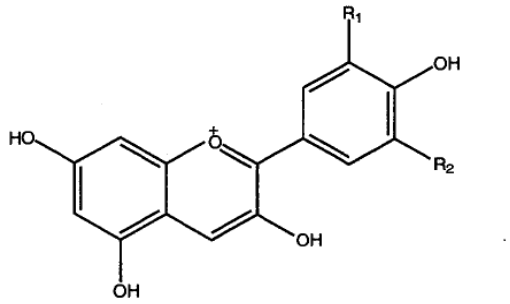
(Table 1.1 continued)

Folate (mg)	6.00
Vitamin A (IU)	54.00
Vitamin E (mg ATE)	0.57

However low bush blueberry, known as wild blueberry is grown in northeastern USA and Canada, and sold mainly as frozen blueberries (Girard and others 2006). The composition of blueberries is shown in Table 1.1. The *Vaccinium corymbosum* (northern high bush) is the common cultivated species. Southern high bush blueberry is a new type of blueberry which is a hybrid of northern high bush and native southern blueberry species. High bush blueberries are large in size with intense dark blue color which increases its fresh market value and suitability for processing. North America is the world's leading producer of blueberries where 60% of the fruit goes to the fresh market and 40% is processed (Trehane 2004).

1.2.1 Anthocyanins

Anthocyanins are naturally occurring water soluble pigments which are glycosides of aglycones of anthocyanidins. Anthocyanins change color depending on the pH of the medium. In acidic solutions, their color changes to red, and in basic medium their color changes to blue while in neutral conditions, anthocyanin has a violet color. The bluish red colors of the blueberries are mainly due to presence of anthocyanins.



Cyanidin R_1 = OH , R_2 = H

Peonidin R_1=OCH_3, R_2 = H

Delphinidin R1=R_2=OH

Petunidin R_1=OCH_3, R_2=OH

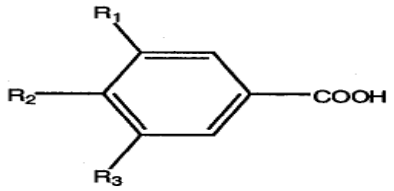
Malvidin R_1=R_2=OCH_3

Figure 1.4 Anthocyanidin structure

There are fifteen different anthocyanins present in blueberry fruit. They are galactosides, glucosides, and arabinosides of delphinidin, cyanidin, petunidin, peonidin and malvidin (Kalt and Dufour 1997; Girardin and others 1996; Haluk and others 1998).

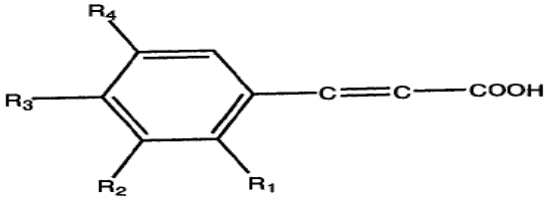
Anthocyanins have antioxidant properties which play an important role in prevention of cardivasular illnesses, cancer and diabetes. There are several factors effecting anthocyanin stability such as pH, temperature, light, oxygen, enzymes, ascorbic acids, sugars, sulfite salts (sulfur dioxide), metal ions and co-pigments. Heating magnitude and temperature have a greater effect on anthocyanin stability (Brunton and others 2010a; Erdman and others 2005).

1.2.2 Polyphenols



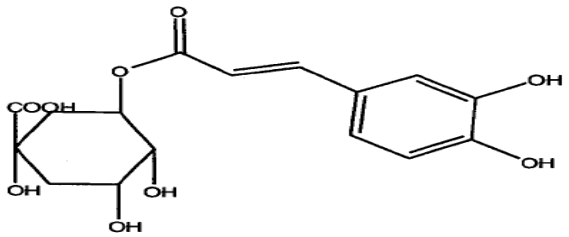
Gallic acid	$R_1=R_2=R_3=OH$
Protocatechuic acid	$R_1=H$, $R_2=R_3=OH$
Vanillic acid	$R_1=H$, $R_2=OH$, $R_3=OCFI_3$
Syringic acid	$R_2=OH$, $R_1=R_3=OCH_3$

Benzoic acid

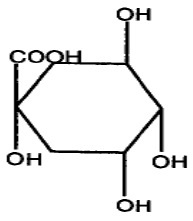


Ferulic acid	$R_1=R_2=H$, $R_3=OH$, $R_4=OCH_3$
p-coumaric acid	$R_1=R_2=R_4=H$, $R_3=OH$
o-coumaric acid	$R_2=R_3=R_4=H$, $R_1=OH$
m-coumaric acid	$R_1=R_3=R_4=H$, $R_2=OH$
Caffeic acid	$R_1=R_4=H$, $R_2=R_3=OH$

Cinnamic acid



Chlorogenic acid



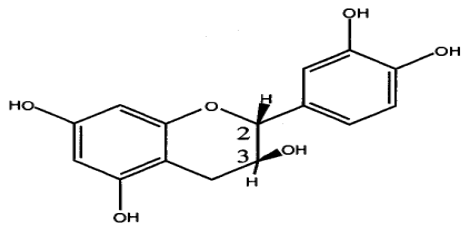
Quinic acid

Figure 1.5 Structures of phenolic acids present in blueberries

The composition of phenolic acids varies between blueberry cultivars. The structures of phenolic acids are shown in Figure 1.5.

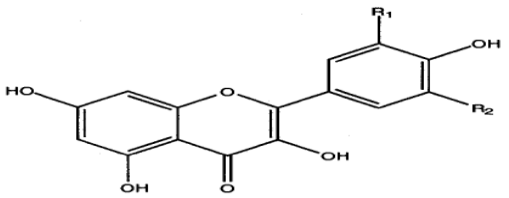
Most of the phenolic acids present in blueberries are derivatives of benzoic acids and cinnamic acids. These phenolic acids are also known as non-flavonoids. Vanillic acid, syringic acid, gallic acid, protocatechuic acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid and ellagic acids are derivatives of benzoic acids (Sellappan and others 2002; Häkkinen and others 1999; Amakura and others 2000), while chlorogenic acid, caffeic acid, ferulic acid, quinic acid, p-coumaric acid, o-coumaric acid and m-coumaric acid are derivatives of cinnamic acids (Sellappan and others 2002; Häkkinen and others 1999). The major phenolic acid present in blueberries is chlorogenic acid (Kalt and others 2000; Wang and Zheng 2003). Phenolic acids are rarely present as free acids and are mostly esterified with other phenolic, acids and sugars.

1.2.3 Flavonoids



(+)-Catechin (2R, 3S)
(-)-Epicatechin (2R, 3R)

Flavanols



Kaempferol	R_1=R_2=H
Quercetin	R_1=OH, R_2=H
Myercetin	R_1=R_2=OH

Flavonols

Figure 1.6 Some structures of flavonoids present in blueberries

Blueberries are rich in flavonoids. Anthocyanins, flavonols, flavones, flavanonols and flavan-3-ols have been reported to be present in blueberries. Flavonoids exhibit antioxidant and anti-carcinogenic properties. According to previous epidemiological studies, high intake of flavonoids provides protection against coronary heart disease, stroke and lung cancer. Quercetin, kaempferol and myricetin are some flavonols present in blueberries (Sellappan and others 2002; Azar and others 1987).

1.2.4 Antioxidant Activity

Blueberry has high antioxidant activity compared with many other fruits. The antioxidant activity of the berries ranges from 8.1-38.3 μmol TE/g. According to the USDA Human Nutrition Research Center, fresh blueberries ranked among the top for having higher antioxidant activity (ORAC 2400 units/ 100g) compared with many fruits and vegetables (Chien and Su 2007). The factors important for antioxidant activity in blueberries are mainly anthocyanin content, phenolic content, maturity and post-harvest storage conditions.

1.2.5 Nutritional and Health Benefits

Diets rich in fruits and vegetables have been known to reduce certain types of cancer, cardiovascular and chronic diseases (Donner and others 2000). Most of these benefits are related to phytochemicals present in fruits and vegetables which contribute to their antioxidant activity. The natural antioxidants present in fruits and vegetables such as anthocyanins, polyphenolics, vitamin C, vitamin E and carotenoids are assumed to have a preventive action against chronic diseases. Antioxidant compound reduce the oxidative damage of free radicals by neutralization. Free radicals can be produced within the human body due to body functions as respiration, and can be induced through environmental conditions as UV radiation, air pollution, and habits such as consumptions of charred food and smoking (Erdman and others 2005). The importance of blueberries should be emphasized

because they have the highest antioxidant capacity compared to 42 fruit and vegetables evaluated. Blueberries are also known as a good source of dietary fiber, calcium, iron, vitamin A and Vitamin C.

1.2.6 Blueberry Juice

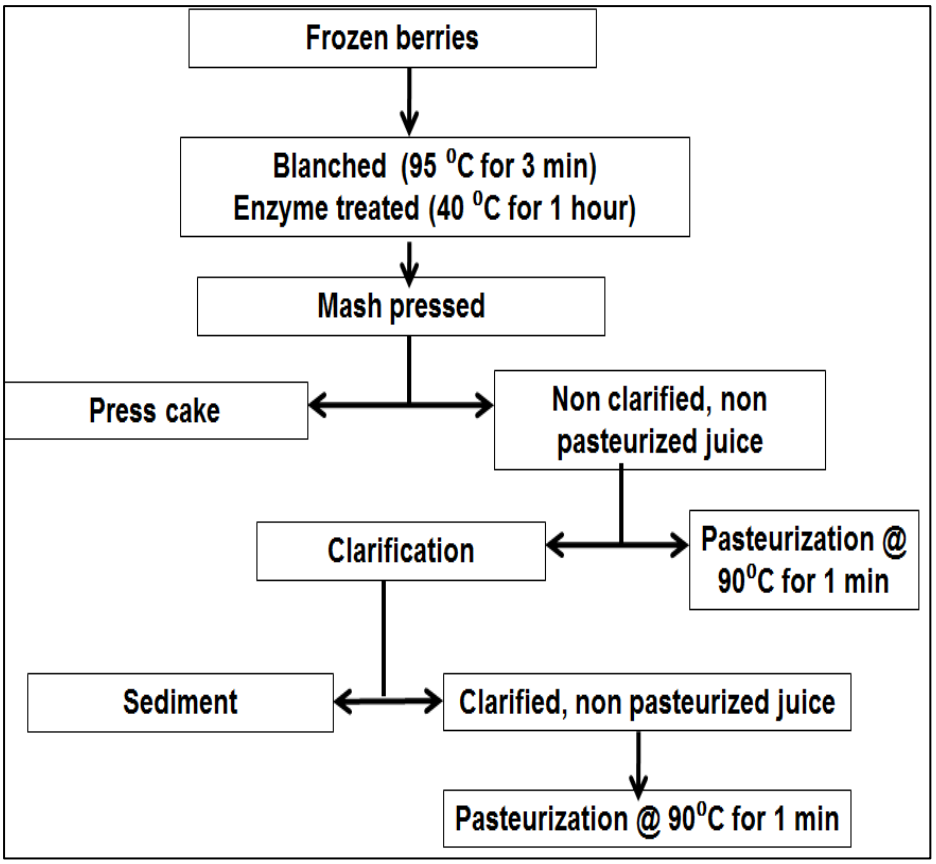


Figure 1.7 Blueberry juice processing (Source: Durst and others 2002)

Blueberry juice is one of the processed products of blueberries. Commercial blueberry juice processing involves the processing steps of blanching, enzyme treatment, clarification and pasteurization (Figure 1.7).

1.3 Marine Oils

Marine fish oils are the main source of long chain omega-3 poly unsaturated fatty acids (Anwar and others 2010). The omega-3 poly-unsaturated fatty acid group includes alpha-linolenic acid (ALA C 18:3), eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6). The chemical structures of EPA and DHA are shown in Figure 1.8.

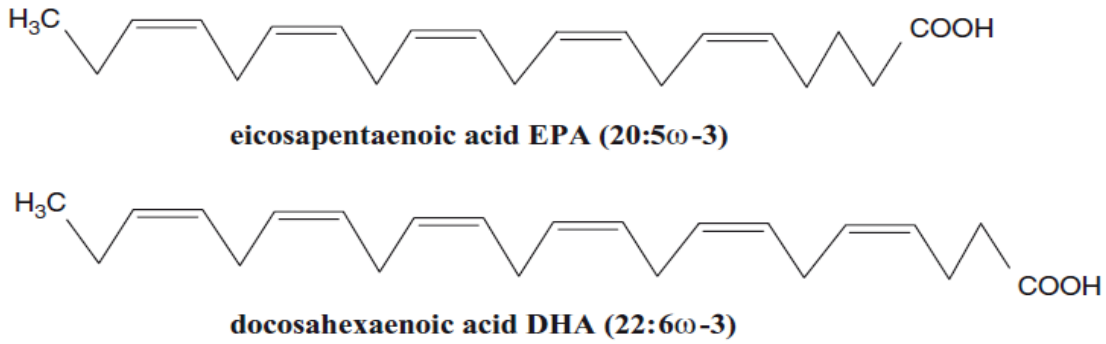


Figure 1.8 Chemical structures of EPA and DHA

1.3.1 Menhaden Oil

Menhaden is a small oily fish which is bright silver in color having a black spot from the gill plate to the tail. Menhaden is found along the Atlantic and Gulf coasts of the United States as two species: the Atlantic menhaden (*Brevoortia tyrannus)* and the Gulf menhaden (*Brevoortia patronus)* (IFFO, 2005). Menhaden is currently used for fish meal and fish oil production. In 2008, more than 608.45 million kilograms of menhaden was harvested in the United States, and in 2005 the Gulf of Mexico had a menhaden oil production of 46528 metric tons (Bechtel and others 2011). The fish meal is used as a feed additive for a variety of domestic animals and fish in aquaculture. The fish oils are used as lubricants, but recent investigation of health benefits associated with fish oil has increased its value. Currently menhaden fish oil has been purified to produce omega-3 enriched health products for human consumption.

1.3.2 Salmon Oil

Salmon oil is an important by product obtained from salmon fish. The salmon head and viscera are used for salmon oil extraction where the salmon head contains 10.9% oil and the viscera contain 2% oil (Behchtel and Wu 2008). Salmon oil is also a rich source of omega-3 long chain fatty acids.

1.3.3 Health Benefits

According to many research findings poly unsaturated fatty acids are associated with a low incidence of coronary heart disease and cancers (Barrow and others 2009). Both EPA and DHA are important for cardiovascular systems.

EPA has an anti-inflammatory response acting as a building block of eicosanoids known to act as cell messengers. Eicosanoids have an effect on blood pressure, blood clotting, immune function, allergic response and gastric secretion. DHA is mainly a structural component of the brain, which relates to brain function (Berger and others 1999; Chan and others 2009; IFFO 2008; Barrow and others 2009).

1.3.4 Lipid Oxidation

Marine fish oils are very sensitive to lipid oxidation due to their highly unsaturated nature. Lipid oxidation of marine oils leads to formation off flavors and odors while degrading important health beneficial compounds such as EPA and DHA.

Lipid oxidation is a free radial reaction which occurs in three distinct stages as initiation, propagation and termination (Colakoglu 2007; McDonald and Min 1996).

- Initiation

Lipid oxidation can be initiated by number of factors including heat, UV and ionizing radiation, peroxides (lipoperoxides and hydrogen peroxides) and metals.

$$RH \xrightarrow{\text{Energy / catalyst}} R. + H.$$

Under these conditions, peroxides are cleaved and formed into alkoxy and hydroxyl radicals. These can act as initiators of subsequent chain reactions (Halliwell and Chirico 1993; Ladikos and Lougovois 1990).

- Propagation

The propagation reaction is primarily a radical chain reaction. During this stage peroxide radicals react with oxygen to form the radical ROO accelerates degradation. In order to prevent this reaction the oxygen level should be very low (Halliwell and Chirico 1993; Ladikos and Lougovois 1990).

$R. + O_2 \longrightarrow ROO.$

$ROO. + RH \longrightarrow ROOH + R.$

- Termination

The propagation reaction continues until the termination reaction occurs. The termination reactions lead to formation of dimers, polymers, ketones and alcohols (Halliwell and Chirico 1993; Ladikos and Lougovois 1990).

$R. + R. \longrightarrow RR$

$ROO. + ROO. \longrightarrow O_2 + ROOR$

$ROO. + R. \longrightarrow ROOR$

1.4 Microencapsulation

Microencapsulation refers to the envelopment of small solid particles, liquid droplets or gases in a coating mainly consisting of polymers. Microcapsules consist of two distinct regions as inner core material and external wall material. Microcapsules can be categorized into two groups based on their morphology; mononuclear capsules where a single core is enveloped by a coating, and aggregates which have many cores surrounded in a matrix (Homayouni and others 2008). Microencapsulation of marine oils prevents contact with

oxygen, metals and exposure to light. Also encapsulation facilitates trapping of off flavor in marine oils while facilitating ease of enrichment in foods (Shahidi and Han 1993).

1.4.1 Wall Materials

Stability of produced fish oil microcapsules depends heavily on wall material and processing conditions. The choice of wall materials for microencapsulation is critical since it determines the initial feed emulsion properties, retention of core material during the process of drying and shelf life of the powders after drying. The main types of wall material used for microencapsulation are carbohydrates (modified and hydrolyzed starch, cellulose derivatives, gums and cyclodextrin) and proteins (whey proteins, caseinates and gelatin) (Shahidi and Han 1993).

1.4.2 Spray Drying

Spray drying is the most commonly used microencapsulation technique in the food industry. Initially oil in water emulsion is prepared and the water phase is converted into a dry powder by spraying the feed into a hot dry air. The typical spray drying process contains several stages including atomization, air contact, evaporation and product recovery. Spray drying is a one step process where drying proceeds until the desired moisture content in the product is obtained (Shahidi and Han 1993). The resident time of particles within the spray drier is less than 30 seconds (Adamiec and others 2002) thus the entire process of dehydration takes only a very short time. The fish oil loading obtained through spray drying ranges from 1 to 60% in weight, and particle size ranges from 10 to 400 μm.

CHAPTER 2 EFFECT OF THERMAL PASTEURIZATION AND ULTRA-SONICATION ON PHYSICO-CHEMICAL PROPERTIES AND MICROBIAL COUNTS OF BLUEBERRY (*VACCINIUM CORYMBOSUM*) JUICE

2.1 Introduction

In recent years, the application of ultrasonication in food processing has been attractive because of its ability to inactivate microorganisms either without altering or having relatively small effect on bioactive compounds, sensory and nutritional properties of food (Heinz and others 2004). Ultrasonication is considered as one of the potential non thermal pasteurization techniques for processing fruit juice (Deeth and others 2003). It is a simple and effective method in retaining original characteristics of fruit juice which may make it more advantageous than thermal pasteurization. The 2001 FDA report on "Kinetics of microbial inactivation for alternative food processing technologies" indicated that ultrasound processing could be used as one of the potential alternative techniques for conventional pasteurization (Roberts and Salleh-Mack 2007). Ultrasound can be divided into three levels based on its frequency ranges: diagnostic ultrasound (1-10 MHz), high frequency ultrasound (100 kHz-1 MHz) and power ultrasound (16-100 kHz) (Bates and Patist 2008).

Inactivation of microorganisms can be explained by the effect of mechanical cavitation generated by ultrasonication. Cavitation alters physical and chemical properties of a food depending on the type of material involved (Cullen and others 2010). Ultrasound waves passed through a liquid medium induces high pressure (compression) and low pressure (rarefaction) wave cycles on the molecules of the liquid medium. Gas bubbles formed within the liquid medium (in our study liquid food) by mechanical cavitation, are then dispersed throughout the liquid. These bubbles continuously grow bigger and reach a condition where they are unstable and begun to collapse (Bates and Patist 2008). The collapsing bubbles generate high temperature hot zones and pressure in the medium (Feng and others 2008).

The high temperature and pressure generated in the liquid medium due to cavitation are last only for a very short period of time (Flint and Suslick 1991). However they are able to damage the cell membranes of microorganisms. The high pressure generated in the medium due to the bubbles collapsing is the main cause of microorganism inactivation. The hot zones are also able to kill microorganisms but their effect is localized. The growth and collapse of the bubbles determine the amount of energy released by cavitation. Low frequency ultrasound, for example 20 kHz, is more effective in inactivating microorganisms because it generates large cavitation bubbles resulting in high temperature and pressure generated in the cavitation zone (Bates & Patist, 2008). Chouliara et al. (2010) have shown that ultrasound can reduce the total viable counts and psychrotrophs in raw and thermally sterilized milk, an effect that last for up to 6 days of storage. Also Ultrasound has shown significant effect on inactivating *E.coli* (Roberts and Salleh-Mack 2007).

Recently blueberries (*Vaccinium corymbosum L.*) have gained much consumer attention due to the presence of health promoting compounds such as polyphenols and anthocyanins. These compounds have antioxidant properties which can neutralize unstable free radicals linked with the development of a number of diseases such as cancer, cardiovascular disease and age related conditions such as Alzheimer's (Cao et al., 1998). According to the USDA database, blueberries have an antioxidant activity of 6552 µmol TE/100g, which is reported as higher than many fruits and vegetables (USDA, 2004). The polyphenols and anthocyanins present in the berries are the major contributors to their antioxidant activity (Durst and others 2002). Anthocyanins are important for blueberry juice in terms of juice color and antioxidant activity and have been shown to have an important role in the prevention of neuronal and cardio vascular diseases, cancer and diabetes (Brunton and others 2010a). Because of seasonality and short shelf life more than fifty percent of harvested blueberries are used for processing into juice, purees and other blueberry products

(NASS 2006). The thermal processing employed in juice production readily degrades anthocyanin pigments affecting juice color and nutritional properties (Guimarães and others 2010). The blueberry juice processing industry desires a fresh like product with natural color and flavor while preserving the nutritional and health compounds initially present in the fruit. Presently, thermal pasteurization is the common method used for blueberry juice processing to provide microbiological stability and extended shelf life (Brownmiller and others 2008). But thermal pasteurization is associated with degradation of anthocyanins and sensory properties. Therefore there is a need for a non-thermal preservation technique or a technique to provide adequate microbial stability which can also preserve the bioactive compounds, nutritional and sensory properties.

The objectives of this study were to: (1) determine the effect of thermal pasteurization and of continuous ultra-sonication processing on the microbiological, chemical and physical properties of blueberry juice, (2) and to estimate the influence of temperature on blueberry anthocyanin degradation kinetics.

2.2 Materials and Methods

2.2.1 Preparation of Blueberry Juice

Locally purchased fresh imported northern high bush blueberries (*Vaccinium corymbosum* L.) were used to produce blueberry juice according to the process flow diagram shown in Figure 2.1. Berries were steam blanched for 2 min and rapidly cooled to 10 °C. After that berries were blended using a mechanical blender (Magic bullet, China) for 1 min, and the mash was centrifuged at 2037.6×g (J2-HC-TB-002 model, Beckman instruments Inc., CA, USA) for 20 min at 4 °C to separate the juice from the solid phase.

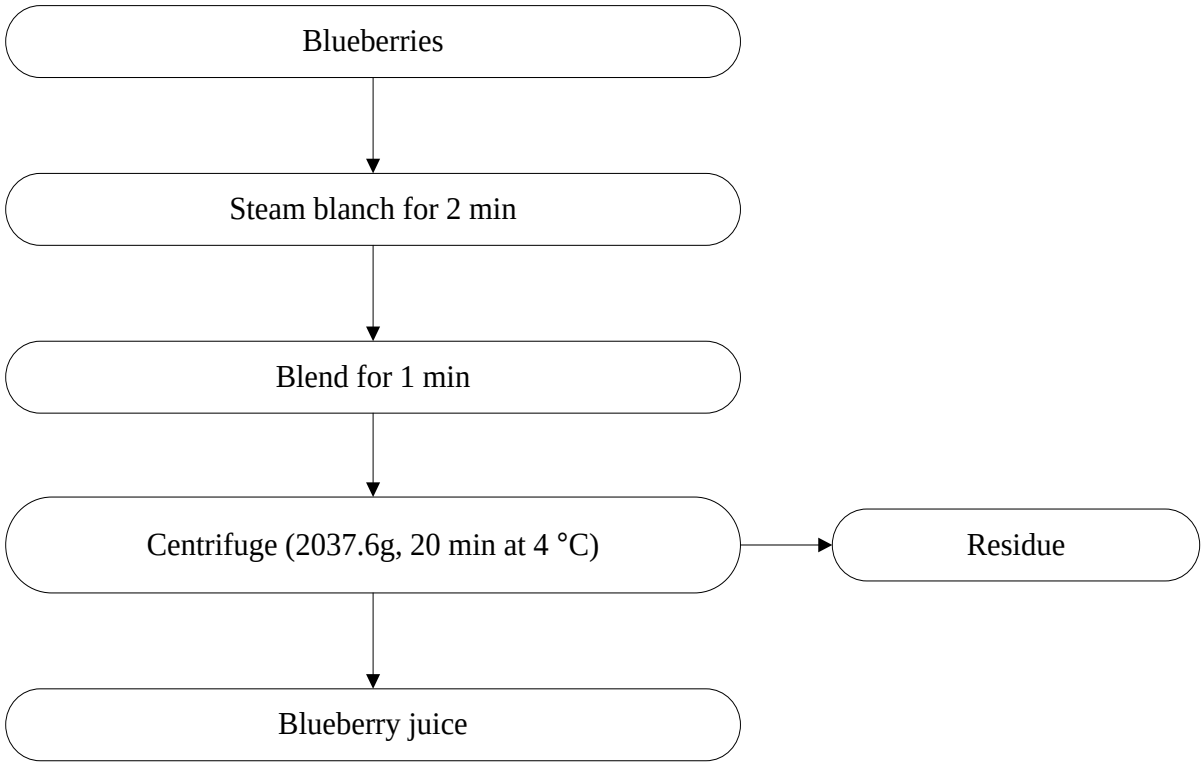


Figure 2.1 Processing flow diagram of juice processing

2.2.2 Thermal Pasteurization Treatments

A 200 mL of blueberry juice (BJ) placed in a 500 ml conical flask container was heated using a water bath (Model 1235 PC, VWR scientific, Singapore) until the temperature of the juice reached 80, 85 or 90 °C. The juice samples were then kept in the water bath with periodical agitation for 1, 2, 3, 4 and/or 5 min at the obtained temperature. Thermally pasteurized (TP) juice samples were immediately cooled in an ice water bath till the temperature of juice samples reached 25^{o}C.

2.2.3 Ultra-sonication Treatments

A continuous ultra-sonication system was used to provide sonication treatments as described by Pérez et al. (2010). The system consists of an ultrasonic processor (Model CPX 500, Cole Palmer Instruments, Vernon Hills, IL, USA) with an ultrasonic probe of 10 mm

diameter and an ultrasonic converter for producing sonic waves at a frequency of 20 kHz (Figure 2.2).

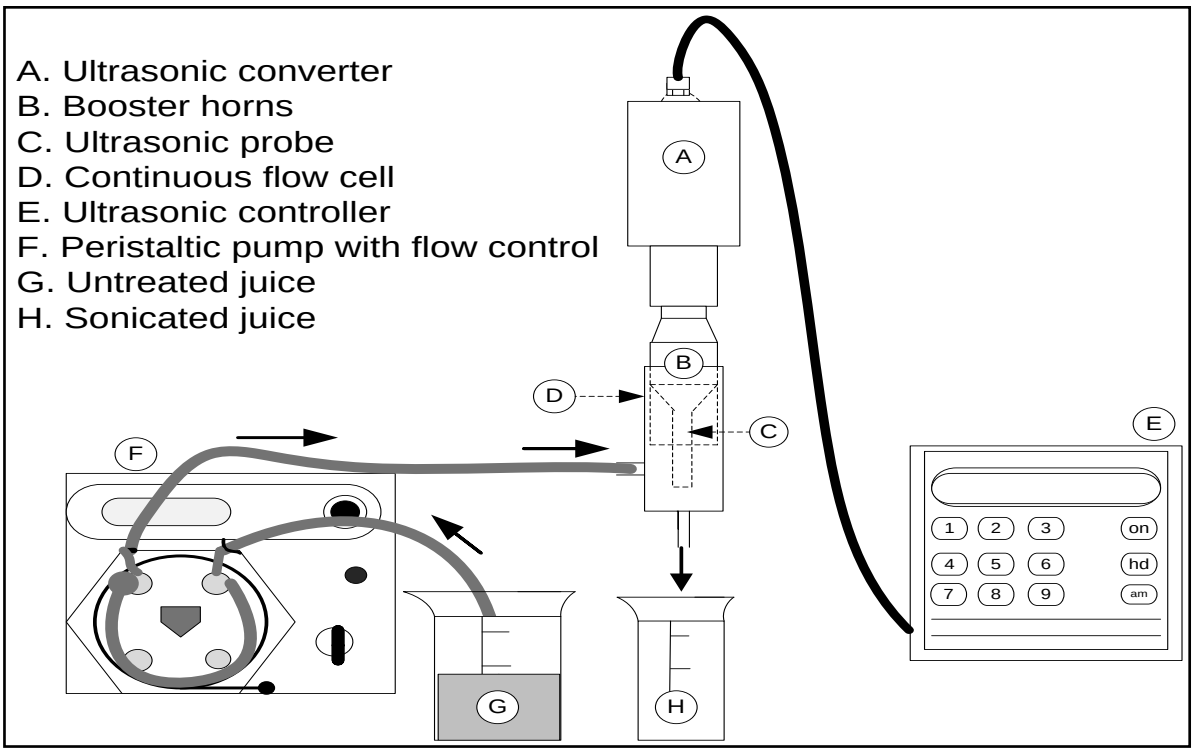


Figure 2.2 Ultra-sonication system used to sonicate blueberry juice

The continuous system was established using a double wall stainless steel cylindrical continuous flow cell (Figure 2.3) which was screwed onto the threaded portion of the ultrasonic probe. The blueberry juice samples (200 mL) were pumped at the rate of 24 mL/min or 93.5 mL/min using a peristaltic pump (Master Flex L/S, Cole Palmer, Vernon Hills, Il, USA) to the continuous flow cell where juice samples were sonicated at 40, 80 or100% amplitude by the ultrasonic processor. A control juice sample was prepared by passing the juice sample through the flow cell without sonication at a rate of 24 mL/min or 93.5 mL/min. Prior to the experiment the continuous flow cell was sanitized using 200 ppm solution of sodium hypochlorite (Sigma-Aldrich co., St Louis, MO, USA). The temperature of all the samples was maintained at 25 ^{o}C using a thermostatic bath during processing.

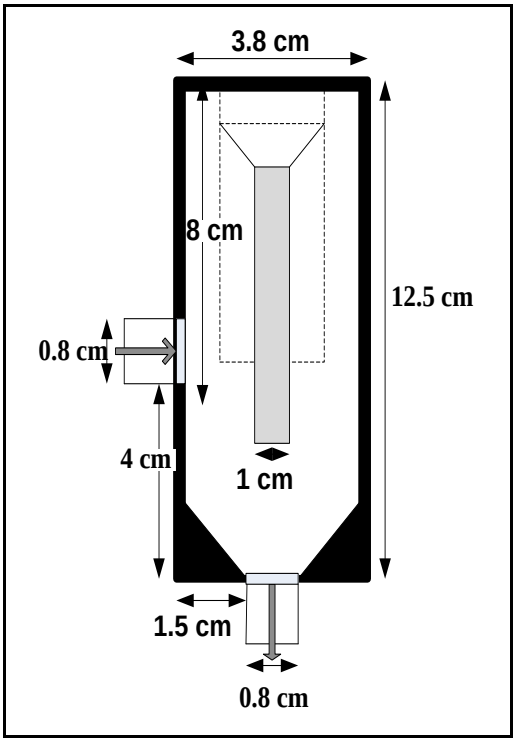


Figure 2.3 The continuous flow cell

2.2.4 Estimation of Applied Energy Rate (Q)

The energy input (E), processing time (t) and treated juice volume (V) were recorded and they were used to calculate the applied energy rate for each ultra-sonication treatment (Eq. 2.1) The calculated Q (W/mL), is a function of power level and time of exposure.

$$Q = \frac{E}{t \times V} \tag{2.1}$$

2.2.5 Microbial Counts

2.2.5.1 Aerobic Plate Count (APC)

Blueberry juices treated by TP, Ultrasonication, and control were analyzed in triplicate for aerobic bacteria by using 3M™ Petri Film™ aerobic count Plate (3M Company, St. Paul, MN.APC provides a general estimate of live aerobic bacteria, analyzed using the technique AOAC 990.12 (2005b) Petri Films are a double film system, wherein the lower film is coated with dehydrated nutrients and water soluble gelling agents and the upper film

contains gelling agents and 2,3,5-triphenyltetrazolium chloride (TTC). One mL of juice was placed on the center of the lower film and then the upper film was placed on the top of the lower film. After gelling (<1 min) petri film plates were incubated for 48 h±3h at 35°C±1°C and the red colonies were counted using the naked eye.

2.2.5.2 Total Coliforms (TC)

All juice samples were analyzed for total coliforms (AOAC 991.14, 2005b) using total coliform Petri Film obtained from 3M Microbiology (St Paul, MN, USA). The Petri Films contain violet red bile (VRB) nutrients, gelling agent and an indicator of glucuronidase activity. As above, 1 mL of juice samples was inoculated in the Petri Films and incubated for 24 h±2h at 35 °C±1 °C and the red colonies with formed gas were counted. The coliforms are considered as gram negative rods which ferment lactose and produce acid and gas. The produced acid changes the pH modifying the gel color into darker red. The gas trapped around red colonies confirms the presence of coliforms.

2.2.5.3 Yeasts and Molds

All of the juice samples were analyzed in triplicates for yeasts and molds (AOAC 997.02, 2005b) using Petri Films (3M Microbiology, St Paul, MN, USA). The Petri Films contain nutrients and a gelling agent favorable for yeast and molds growth. Yeast are typically indicated by small blue-green colonies with diffuse edges and center foci while molds are identified by large variably colored colonies with diffuse edges and center foci. One mL of juice was used as the inoculant as explained previously. Yeasts and molds colonies were counted after an incubation period of 3 to 5 days at room temperature.

The APC, TC and yeasts and molds colonies were counted and reported as CFU/mL using Eq. 2.2.

CFU/mL = [number of colonies * dilution factor] (2.2)

Aliquot plated (1mL)

2.2.6 Total Anthocyanin Content (TAC)

The total monomeric anthocyanin content of the blueberry juice was determined by using the pH differential method of AOAC 2005.02 (AOAC, 2005a). Juice samples were separately diluted with buffer solutions (Sigma-Aldrich Co., St Louis, MO, USA) of KCl (0.025 M, pH 1.0) and CH_3COONa (0.4 M, pH 4.5) according to a pre-determined dilution factor (Dilution factor 10). The absorbance of these prepared dilutions was measured at 520 nm and 700 nm using a spectrophotometer (Thermo Fisher Scientific, Vernon Hills, IL). The absorbance value (*A*) was calculated as follows (Eq.2.3).

$$A = \left(A_{520} - A_{700}\right) pH_{1.0} - \left(A_{520} - A_{700}\right) pH_{4.5} \quad (2.3)$$

Where A_{520} is the absorbance value at 520 nm in pH 1.0 or pH 4.5 buffers; A_{700} is the absorbance value at 700 nm in pH 1.0 or pH 4.5 buffers.

The monomeric anthocyanin content of the juice samples was calculated using Eq. 2.4, and was expressed in mg/L of cyanidin-3-glucoside equivalents.

Monomeric anthocyanin pigment concentration (mg/L) =

$$\frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (2.4)$$

Where MW is the molecular weight of cyanidin-3-glucoside (449.2 $gmol^{-1}$), DF is the dilution factor (10), ε is the molar extinction coefficient (26900 $Lmol^{-1}cm^{-1}$), and l is the path length of the cuvette in cm (1 cm).

2.2.7 Total Phenol Content (TPC)

The total phenol content of juice samples was analyzed according to Folin-ciocalteu method (Singleton and Sinkard 1997). Twenty µl of diluted juice samples (1:10) was prepared with 1.58 ml of water and 100 µl Folin-ciocalteu reagent (Sigma-Aldrich co., St Louis, MO, USA) in a cuvette. The juice mixture was shaken for 30 sec and let stand for 5 min, and a 300 µl solution of saturated sodium carbonate was added to the mixture. The mixtures were placed in cuvettes at 20°C for 2 hours and absorbance was determined at 765 nm using spectrophotometer (Thermo Fisher Scientific, Vernon Hills, IL) using a blank solution prepared with 20 µl of distilled water. The results were expressed as mg/L of gallic acid equivalent using a gallic acid standard curve prepared using gallic acid concentrations of 0, 50, 100, 150, 250 and 500 mg/L.

2.2.8 Antioxidant Activity (AA)

The DPPH radical scavenging assay was used to determine the antioxidant activity of the juice samples according to the method of Arlorio et al. (2009). A 700 µl of 100 µM DPPH (Sigma-Aldrich co., St Louis, MO, USA) solution prepared in methanol (Sigma-Aldrich Co., St Louis, MO, USA) was mixed with an equal volume of juice. The mixture was shaken vigorously and kept in a dark room at room temperature for 30 min. The juice mixture was placed in a spectrophotometer (Thermo Fisher Scientific, Vernon Hills, IL) and absorbance was measured at 515 nm (Ab sample). A solution of 700 µl 100 µM DPPH and 700 µl distilled water was prepared and absorbance was measured at 515 nm (Ab control). Methanol was used as a blank for the spectrophotometer analysis. The antiradical activity of the samples was calculated as inhibition percentage using Eq. 2.5.

$$\text{Inhibition percentage (\%)} = \frac{\text{Ab control} - \text{Ab sample} \quad \times 100}{\text{Ab control}} \tag{2.5}$$

The procedure was also repeated for the trolox standard curve using trolox concentrations of 4, 8, 12, 16 and 20 µg/mL and the data were expressed as mmol/100 mL trolox equivalent.

2.2.9 Total Soluble Solids, pH, and Titratable Acidity

The juice samples were analyzed for total soluble solids (TSS), pH and titratable acidity. The total soluble solids of juice samples were determined at 20 °C using a digital refractometer (Model AR 200 Reichart Analytical Instruments, NY, USA). Blueberry juice samples were separately placed on the lens of the refractometer, and analyzed at 20°C. Results were reported as °Brix values. The pH of the juice was measured using a bench top pH meter (Symphony, VWR Scientific 5B70P, PA, USA). Titratable acidity was determined by titrating a 10 g aliquot of blueberry juice sample to pH 8.2 using 0.1 N NaOH (Sigma-Aldrich co., St Louis, Mo, USA). The results were expressed as percent total organic acid on the basis of citric acid.

2.2.10 Measurement of Juice Color

The Color of the juice samples were measured in triplicate using a Lab Scan XE Colorimeter (Hunter Associates Laboratory, INC. Resbon, VA, USA) and was reported in CIE LAB color scale (L^*, a^* and b^* values). The instrument was calibrated initially with black and white standard tiles. Four grams of blueberry juice were weighed and placed in a hexagonal plastic container. Five containers were stacked together to prevent disturbance from the black base. Chroma and hue angle value were calculated using Equations (2.6) and (2.7), respectively. The negative hue angle values were converted into positive values by adding 180°to make them fall in the 90° – 180° quadrant (+b* = yellow; -a* = green).

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \qquad (2.6)$$

Hue angle = $\tan^{-1}(\frac{b^*}{a^*})$ (2.7)

2.2.11 Degradation Kinetics of Anthocyanins

A first order (Eq. 2.8) model was used to describe the anthocyanins degradation kinetics according to Guimarães et al. (2010).

$C_t = C_o \exp(-kt)$ (2.8)

$t_{1/2} = -\ln 0.5 / k$ (2.9)

Where C_o is the initial anthocyanin content (mg/L cya-3-glu); C_t is the anthocyanin content (mg/L cya-3-glu) after heating at 80, 85 or 90 ^oC for 1, 2, 3, 4 and/or 5 min. (t) The first-order degradation rate constant is represented as k and $t_{1/2}$ is half life time (Eq. 2.9).

The logarithms were taken on both sides of Eq. 2.8 (Eq. 2.10).

$$\ln C = \ln C' - kt \quad (2.10)$$

Eq. 2.10 was rearranged as Eq. (2.11)

$$\ln\left(\frac{C}{C_t}\right) = -kt \quad (2.11)$$

A plot of $\ln\left(\frac{C}{C_t}\right)$ versus t was constructed to determine k values and correlation coefficients. The k value from the first order model was used to estimate the half- life of anthocyanin using Eq 2.9. ln(k) values were plotted against 1/T and the activation energy for anthocyanin degradation was obtained with the Arrhenius model (Eq. 2.12). The magnitude of E_o was calculated as the slope of the plot multiply by the gas constant.

$$\ln k = \frac{-E_o}{RT} + \ln k_o \quad (2.12)$$

Where k_o is the frequency factor (min^{-1}); k is the degradation rate constant; E_o is the activation energy ($kJmol^{-1}$); R is the universal gas constant (8.314 $Jmol^{-1}K^{-1}$); and T is the absolute temperature (K).

The effect of temperature on the rate of reaction was alternatively characterized by the temperature coefficient (Q_{10}). The change in the anthocyanin degradation can be explained by Q_{10} when the temperature increases by 10 °C (Eq. 2.13).

Anthoycyanin degradation rate constant at 80 °C and 90 °C were used to calculate Q_{10} value using the Equation 2.13.

$$Q_{10} = \left(\frac{K_{at}80^{\circ}C}{K_{at}90^{\circ}C}\right)^{(10/90^{\circ}C-80^{\circ}C)} \quad (2.13)$$

2.2.12 Statistical Analysis

All of the treatments and analyses were conducted in triplicate. One way analysis of variance (ANOVA) following a Dunnett Test was carried out to determine the difference between treatment means with the control. Means of treatment combinations of (thermal processing temperature and time, and ultra-sonication flow rate and amplitude) were separately analyzed using a 2-factorial randomized block design (RCBD). The treatment main and interaction effects were obtained using the post hoc test of Tukeys Studentized Range Test (SAS version 9.2, SAS institute INC., Cary, NC, USA). The statistical significance level was set to $\alpha = 0.05$.

2.3. Results and Discussion

2.3.1 Applied Energy Density

The applied energy density (Table 2.1) designates that the application of total sonication energy per mL of juice during the ultrasonication process (Ashokkumar and others 2010). The resident time for sonicating juice reduces with high flow rate and sonication energy rate increases with increasing sonication intensity (amplitude).

Table 2.1 Applied energy density to blueberry juices during ultrasonication

Amplitude	Applied energy rate (W/mL) at 24 mL/min	Applied energy rate (W/mL) at 93.5 mL/min
40%	13.88±2.45	3.64 ±1.75
80%	43.45±4.12	10.58 ±1.32
100%	73.60±5.35	19.74 ±2.03

2.3.2 Microbial Counts

Until the occurrence of major outbreaks related to *E.coli* 0157:H7 and salmonella in non-pasteurized orange and apple juices, it was widely accepted that most low pH, high acid foods do not cause food poisoning outbreaks (Parish 1997; Feng and others 2009). This was mainly based on the assumption that organic acids have inhibitory and antimicrobial activity over food borne pathogens. But the outbreaks have shown that the acidity and low pH condition does not assure microbial safety in these fruit juices. After these incidences, FDA required processors to achieve a 5-log reduction in the number of most resistant pathogens in the finish products (USFDA 2001).

Most juice processors use thermal pasteurization to eliminate pathogenic microorganisms. The aerobic plate count, total coliforms, yeasts and mold counts are used to monitor the effectiveness of the pasteurization technique in fruit juices. Table 2.2 shows microbial counts of APC, TC, yeast and mold for blueberry juice treated with thermal pasteurization (TP). All selected temperatures (80, 85 and 90 °C) showed reduced counts ($p \leq 0.05$) of APC, TC, yeasts and molds compared to untreated juice. The reduction was greater with increased temperature and heating time.

Table 2.2 Aerobic plate count (APC), total coliforms (TC) and yeast and mold counts of Untreated and treated blueberry juice with thermal pasteurization (TP) according to temperature and time

Treatment	APC (CFU/mL)	TC (CFU/mL)	Yeast (CFU/mL)	Molds (CFU/mL)
Untreated	30.67±0.47[a]	18.00±0.82[a]	16.00±2.43[a]	1±0.22[a]
TP 80°C/1min	14.33±1.94[b]	6.33±0.47[b]	1.00±0.02[b]	ND
TP 80°C/2min	9.67±0.47[c]	4.33±0.47[c]	ND	ND
TP 80°C/3min	2.33±0.47[d]	ND	ND	ND
TP 80°C/4min	1.00±0[d]	ND	ND	ND
TP 80°C/5min	ND	ND	ND	ND
TP 85°C/1min	1.67±0.47[d]	0.67±0.47[d]	ND	ND
TP 85°C/2min	0.67±0.47[d]	ND	ND	ND
TP 85°C/3min	ND	ND	ND	ND
TP 85°C/4min	ND	ND	ND	ND
TP 85°C/5min	ND	ND	ND	ND
TP 90°C/1min	0.67±0.47[d]	ND	ND	ND
TP 90°C/2min	ND	ND	ND	ND
TP 90°C/3min	ND	ND	ND	ND
TP 90°C/4min	ND	ND	ND	ND
TP 90°C/5min	ND	ND	ND	ND

ND – not detected. Values are means and standard deviations of triplicate determinations. [a-d]means with different exponents in each column are significantly different ($p \leq 0.05$).

Table 2.3 Aerobic plate count (APC), Total coliforms (TC) and yeast and mold counts of Untreated and treated blueberry juice with continuous ultra-sonication (US) flow rate and amplitude.

Treatment	APC (CFU/mL)	TC (CFU/mL)	Yeast (CFU/mL)	Molds (CFU/mL)
Untreated	30.67±0.58[a]	18.00±1.15[a]	16.00±2.43[a]	1±0.22[a]
US control (24mL/min)	31.17±0.15[a]	19.00±1.15[a]	15.00±2.03[a]	1±0.22[a]
US control (93.5 mL/min)	30.03±0.23[a]	19.03±0.32[a]	17.02±2.12[a]	1±0.22[a]
US 24mL/min/40A	12.00±1.73[b]	2.00±0.58[b]	2.00±0.07b	1±0.21[a]
US 24mL/min/80A	5.67±0.58[d]	1.33±0.58[b]	ND	ND
US 24mL/min/100A	3.33±0.75[e]	ND	ND	ND
US 93.5 mL/min/40A	8.67±0.58[c]	1.67±0.1[b]	1.00±0.03[c]	ND
US 93.5 mL/min/80A	3.67±0.58[e]	ND	ND	ND
US 93.5 mL/min/100A	1.33±0.58[f]	ND	ND	ND

ND – not detected. Values are means and standard deviations of triplicate determinations. [a-f]means with different exponents in each column are significantly different ($p \leq 0.05$).

Although the high sugar content of blueberries is often subjects them to mold attacks, mold counts in the final juice prior to pasteurization was lower than expected. This may have been due to blanching. Except for the time temperature combinations of 80°C/ for1 and 2 min, and 85 °C for 1 min, the thermal treatments showed zero counts for total coliforms. Commercial thermal pasteurization of blueberry juices is done by using a time temperature combination of 90°C/60 sec or 90°C/90 sec (Brownmiller and others 2008).

The ultrasound treatment employed using a continuous system (Table 2.3) showed an increased reduction of microbial counts of APC, TC, yeasts and mold with increasing flow rate and amplitude ($p < 0.05$). The flow rate of 93.5 mL/min shows a significant increase in reduction compared to flow rate of 24 mL/min. This may be due to close exposure to the ultrasonic probe compared to the slower flow rate of 24 mL/min. Increase of ultrasound

intensity (amplitude) showed greater reduction in all TPC, TC, yeast and mold counts ($p < 0.05$). This reduction is in agreement with previous research work done by Adekunte et al. (2010) about the effect of sonication on yeast inactivation in tomato juices. They observed an increase in yeast inactivation with increased amplitude and processing time in batch processing conditions. Also they stated that sonication alone can achieve a 5-log reduction in yeast at moderate temperatures. Yeast inactivation was assumed mainly to be due to the combined effect of physical and chemical mechanisms occurring during cavitation. Since yeast cells are relatively rigid and not easy to rupture by cavitation effect, the proposed inactivation is assumed to be mainly due to formation of free radicals (hydrogen peroxide) and release of intracellular protein. Many previous research findings show greater reduction of microorganisms with increase in ultrasound intensity (Jong and Villamiel 2000; Hoover 2000; Chen and others 2009). The sonication control treatments conducted for both flow rates of 24 mL/min and 93.5 mL/min showed the effect on microbial inactivation was mainly due to the sonication effect. The sonication controls showed similar microbial counts as untreated juices.

Although APC counts in ultrasound treated juices show significant reduction, none of the treatments yielded zero counts while many thermal treatments did. According to D`Amico et al. (2006) continuous flow sonication treatment using 20 kHz at 100% amplitude combined with mild heat (57 °C) for 18 min showed a 5-log reduction in total aerobic bacteria present in raw milk and a 6-log reduction in *E. coli* O157:H7 in apple cider. Similarly the effect of ultrasound is more pronounced when combined with heat (Mckellar and others 2003; Mcclements 1995; Chen and others 2007a). A possible explanation for this observation is ultra-sonication makes microbes more susceptible for inactivation by other inhibitory mechanisms. Although an increase of temperature leads to an increase in the number of cavitation bubbles, the collpase of these bubbles is hindered by the increased

vapor pressure (Jong and Villamiel 2000). Therefore cavitation is higher at moderate temperatures. Heinz et al. (2003) demonstrated that ultrasound treatment at moderate temperatures can provide an identical rate of bacterial inactivation as conventional thermal processing.

TC counts (Table 2.3) showed a better reduction with ultrasound, where treatment combinations of flow rate and intensity 24 mL/min and 100% amplitude; 93.5 mL/min 80% and 100% amplitude showed zero counts similar to many thermal treatments. Based on previous research findings gram negative bacteria such as *Escherichia coli* are more succeptilble to sonication inactivation compared to gram positive bacteria. This could be mainly due to the presence of thicker cell walls and a more tightly packed layer of peptidoglycan in gram positive bacteria, which cannot be ruptured by sonication (Mckellar and others 2003). However O`Brien et al. (1991) argued that increased reduction related to gram negative bacteria may be due to the damage on the inner (cytoplasmic) cell membrane because the percentage killed by ultrasound energy cannot be related to the cell morphology. It is belived that pathogenic *E.coli* may survive in acidic environement such as fruit juices. Bluberry juices have a pH value of 3.12, which might enhance acid resistance and the survival of this pathogen in juices. Previous research findings have shown that *E.Coli O157:H7* can survive in concentrates of apple, orange,pineapple and white grape juice up to 12 weeks (Gombas and others 2003). Sonication showed a greater reduction of TC with increasing amplitude (Table 2.3). This observation is in accordance with the research work done by Bourke et al. (2009). They studied the effect of ultrasound inactivation on two strains of *E.coli* and found a greater than 5-log reduction with increasing level of amplitude for a processing time of less than 15 min. The results of that study indicated that ultrasound alone can inactivate acid tolerant *E.coli* effectively in fruit juices without the use of extra heating (Bourke et al., 2009; Roberts and Saleeh-Mack 2007).

Most of the previous research work employed a batch method of sonication where microbial inactivation occured mainly due to a caviation effect and to a lesser extent by generated heat. But continuous flow ultrasound does not generate a significant amount of heat compared to the batch method, where the cavitation effect is exclusively involved in microbial inactivation. The microbial inactivation owing to sonication is mainly ascribed to the physical effect of cavitation, mechanical and micromechanical shocks, and chemical effect of free radical formation in sonicated juices (Adekunte and others 2010; Chen and others 2007a). Although sonication alone can cause microbial inactivation, it might require greater processing time or multiple runs of continuous flow processing. Many previous research studies showed that sonication at lethal temperatures (thermo-sonication) or pressure (Manosonciation) or with the presence of both temperature and pressure (mano-thermo-sonciation) provide a greater effect of inactivation when compared to sonication only treatment. This will enable shorter processing time perhaps resulting in greater juice quality (Feng and others 2009); Heinz and others 2004; Butz and Tauscher 2009).

2.3.3 Total Anthocyanin Content (TAC), Total Phenol Content (TPC) and Antioxidant Activity (AA) of Untreated and Treated Blueberry Juices

Anthocyanins, bioactive compounds, found in blueberries provide desirable reddish purple color in the he fruit juice. They are highly unstable and can degrade and lose their bioactive properties during processing because of conditions including pH, temperature, light, oxygen, and the presence of enzymes and metallic ions (Brunton and others 2010b). The effect of TP on blueberry juice TAC content is shown in Table 2.4. The control (untreated) blueberry contained 408 mg/L of anthocyanin (cyanidin-3-glucoside equivalents). Amakura and others (2000) have reported that anthocyanin content of high bush blueberries ranges from 25 to 495 mg/100g.

Table 2.4 Total anthocyanin content, total phenol content and antioxidant activity of thermally pasteurized and untreated blueberry juice

Treatment	TAC	TPC	AA
Untreated (Control)	408.29±20.39^{a}	1918.61±28.19^{f}	5.08±0.07^{g}
TP 80°C/1min	370.67±13.19^{ab}	2119.61±44.22^{d}	5.90±0.05^{bc}
TP 80°C/2min	366.54±06.85^{ab}	2293.83±13.00^{c}	5.60±0.08^{e}
TP 80°C/3min	351.85±17.53^{b}	2551.94±68.46^{a}	5.40±0.09^{f}
TP 80°C/4min	326.46±12.75^{cd}	2107.50±65.49^{d}	5.85±0.06^{cd}
TP 80°C/5min	312.95±16.21^{de}	2035.28±63.66^{de}	6.21±0.08^{a}
TP 85°C/1min	365.55±10.01^{ab}	2257.50±41.38^{c}	5.78±0.12^{de}
TP 85°C/2min	340.66±09.90^{b}	2093.61±89.38^{de}	5.67±0.12^{e}
TP 85°C/3min	335.99±12.71^{b}	2160.28±42.28^{d}	5.84±0.13^{cd}
TP 85°C/4min	317.30±15.98^{de}	2057.50±41.65^{e}	5.91±0.09^{bc}
TP 85°C/5min	305.28±8.34d^{ef}	2021.39±55.09^{e}	6.23±0.07^{a}
TP 90°C/1min	358.39±12.79^{b}	2263.06±50.23^{c}	5.90±0.09^{bc}
TP 90°C/2min	336.05±11.69^{bc}	2429.72±87.57^{ab}	5.62±0.08^{e}
TP 90°C/3min	329.84±19.00^{cd}	2332.50±48.05^{b}	6.04±0.09^{b}
TP 90°C/4min	304.79±11.03^{ef}	2235.28±48.08^{c}	6.23±0.10^{a}
TP 90°C/5min	297.09±07.57^{f}	2032.50±29.76^{e}	6.32±0.14^{a}

Values are means and standard deviations of triplicate determinations. $^{a-g}$Means with different exponents in each columns are significantly different ($p < 0.05$).

TAC = total anthocyanin content (mg of cyaniding-3-glucoside equivalents /L of juice (cyaniding-3-glucoside equivalents)); TPC = Total phenol content (mg of gallic acid (GA) /L of juice (GA) equivalents)); AA = Antioxidant activity (mmol of trolox (TE)/100 mL of juice (trolox (TE) equivalents).

TP juices had significantly lower levels of TAC than the untreated juice, except TP 80°C/1 min and TP 80°C/2 min. This study showed that the reduction of TAC significantly ($p \leq 0.05$) increased with increasing pasteurization temperature and time. The similar effects of

pasteurization temperature and time on anthocyanin content of strawberry and blackberry purees were observed by Carle et al. (2007); Buckow et al. (2010); Brunton et al. (2010a). They reported that significant anthocyanin reductions in thermally processed strawberry and blackberry purees compared to un-processed puress. Brunton et al. (2010a) have suggested that degradation of anthocyanin is mainly related to opening of its pyrilium ring and formation of chalcone. Chalcone formation is known as the first step of anthocyanin degradation and then the degradation continue and forms compounds such as phloroglucinaldehyde and protocatechuic acid (Carle and others 2007). No significant difference in TAC content was observed between untreated blue berry juice and the juice treated with ultrasonication (US) (Table 2.5), which indicated that ultrasonication treatments had not affected on TAC content of blue juices.

Table 2.5 Total anthocyanin content, total phenol content and antioxidant activity of untreated and treated blueberry juice with ultra-sonication

Treatment	TAC[1]	TPC[2]	AA[3]
Untreated	408.29±21.6^{a}	1918.61±31.55^{e}	5.08±0.07^{d}
US 24mL/min/40A	406.62±31.93^{a}	2068.61±67.87^{d}	5.37±0.09^{c}
US 24mL/min/80A	400.77±36.00^{a}	2199.17±22.05^{c}	5.50±0.07^{c}
US 24mL/min/100A	396.60±27.43^{a}	2243.61±17.35^{b}	5.80±0.11^{b}
US 93.5 mL/min/40A	401.61±23.67^{a}	2129.72±37.58^{cd}	5.70±0.12^{b}
US 93.5 mL/min/80A	397.26±18.23^{a}	2315.83±60.09^{b}	6.01±0.11^{a}
US 93.5 mL/min/100A	393.58±17.45^{a}	2532.50±74.07^{a}	6.11±0.14^{a}

Values are means and standard deviations of triplicate determinations. $^{a-d}$means with different exponents in each column are significantly different ($p < 0.05$).

US = ultrasonication; TAC = total anthocyanin content (mg of cyaniding-3-glucoside equivalents /L of juice (cyaniding-3-glucoside equivalents)); TPC = Total Phenol content (mg of Gallic acid (GA) /L of juice (GA) equivalents)); AA = Antioxidant activity (mmol of trolox (TE)/100 mL of juice (trolox (TE) equivalents)

A similar observation found by Pérez et al. (2010) for blackberry juice when the juice was treated with a continuous flow ultra-sonication system, the TAC content was not affected. Cullen et al. (2009a) reported that ultrasonicated blackberry juice retained a significant amount of anthocyanins (>94%) at the maximum ultrasonication treatment condition (100% amplitude for 10 min). Brunton et al. (2009a) and Brunton et al. (2010b) have reported that about 5% degradation of TAC in fruit juices can occur when fruit juices are processed under extreme non-thermal processing conditions such as sonicating a fruit at 100% amplitude or pressurizing at 500 MPa. However, our study showed that ultrasonication treatments retained TAC in the blueberry juices better than the thermally pasteurized juices regardless of the ultrasonication conditions.

The effect of TP with different heating times and temperature on the TPC of blueberry juices is shown in Table 2.4. The control, the juice treated with TP 80°C/1min,TP 80°C/5min, TP 85°C/4min, TP 85°C/5min, and TP 90°C/5min had lower TPC than juices treated with other TP conditions. The juices treated at 80°C for 1 min and for 3 min had higher TPC than those treated at 80°C for 4 or for 5 min. But the juices treated at 85°C and 90°C show graduation reduction in TPC with increased heating temperature and time.

In general, temperature has a detrimental effect on phenols. Chlorogenic acid, the main phenolic compound present in blueberries has been shown to decrease with heating time and temperature in citrus juices (Chen and others 2007b). The esterified bond of chlorogenic acid is assumed to be cleaved by heat treatment. The effect of US on TPC is shown in Table 2.5. The TPC content of US treated juices significantly increased with flow rate and amplitude ($p \leq 0.05$). All of the US juices have a higher content of TPC than the untreated juice. The highest TPC content was yielded by the treatment of US 93.5 mL/min at 100% amplitude resulting in a having TPC content of 2532mg/L gallic acid equivalent. The sonication increases the TPC in the juice by extracting the bound phenolic present in the

suspended particles (Brunton et al., 2009a; Buchert and others 2004; Brunton et al., 2009b). Extraction of phenolic compounds using ultrasonication showed that sonication has a greater ability to extract phenolic compounds from berries. A study conducted by Chen et al. (2008a) found increased TPC with increasing ultrasonic treatment time and power (Chen and others 2008b).

Table 2.4 shows the AA of untreated and TP blueberry juice measured on the basis of trolox equivalent. TP juices have higher AA than untreated juice. AA of TP juices slightly decrease followed by a significant rise with heating time. Previous research studies have showed that AA of blueberry juices correlated with the polyphenols and anthocyanin content **(**Wang and Zheng 2003)**.** The initial reduction of AA may be due to the heat degradation of anthocyanins present in the juices. The subsequent increase of AA with increased heating time could be attributed to the formation of Maillard browning compounds within the juice. Many researchers have demonstrated the formation of Maillard browning products in thermally pasteurized juices underwent. Furthermore Maillard browning products, especially melanoidin pigments have antioxidant activity, which make them able to scavenge radicals and chelate metal ions (Gonzalez and others 2010; Toledo and Yilmaz 2005).

In the present study (Table 2.4), AA in TP juices increased with increased temperature and heating time can be attributed to increased formation of Maillard browning products. Maillard browning product formation increases with increased temperature and heating time (Agucilò-Aguayo and others 2009b). HMF (5-hydroxymethyl furfural), an intermediate compound of Maillard browning is widely used as an indicator of Maillard reactions. HMF is generally not present in fresh juices (Bozkurt and others 1999), and formation is induced by thermal processing and storage. Aguiló-Aguayo et al., (2009a) reported increased concentration of HMF in heat treated strawberry, tomato and watermelon juices (Jaeger and others 2010).

Ultrasound treated juices (Table 2.5) do not produce browning compounds as thermal processing (Gòmez-Lòpez and others 2010; Cullen and others 2010). Hence the increased AA of US treated juices with increased flow rate and amplitude can be presumed due to extraction of bound polyphenols and anthocyanin from suspended juice particles.

2.3.4 pH, Titratable Acidity and °Brix

No significant difference in pH, titratable acidity or brix ($p >= 0.05$) was noted for any of the treatments (Table 2.6 and 2.7). These results are in agreement with the many previous research findings, where ultrasound processing was shown to have no significant effect on TA, pH and °Brix regardless of ultrasonic intensity (amplitude) and processing time (Kim and others 2004; Cullen and others 2009b; Brunton and others 2010b; Chen and others 2007a; Cullen and others 2008). Also in these studies thermal pasteurization did not show any significant effect on juice TA, pH and °Brix.

2.3.5 Juice Color

Juice color is important for consumer's sensory acceptance. A bright deep red to purple colour is desired in blueberry juices. Also juice deep red color is related to the anthocyanin content in the juices. The L* value is closely associated with the browning in juice which results in a darkening of the color and a decrease in the L^* value (Cullen and others 2009b).

The L* value of TP juices in the present study decrease with increasing temperature and heating time (Table 2.8) indicating the formation of dark color Maillard browning pigments. Hendrick et al. (2007) reported increased degradation of color with increasing temperature in tomato puree and strawberry juices. In previous studies, formations of browning pigments in thermally processed juice are explained by the increased formation of HMF (Aguiló-Aguayo and others 2009a).

Table 2.6 pH, titratable acidity and total soluble solids (°Brix) of Untreated and treated blueberry juice with thermal pasteurization according to temperature and time

Treatment	pH	Titrable acidity[1]	°Brix
Untreated	3.14±0.01[a]	0.84±0.01[a]	11.1±0.1[a]
TP 80°C/1min	3.12±0.01[a]	0.84±0.03[a]	11.0±0.2[a]
TP 80°C/2min	3.12±0.02[a]	0.81±0.03[a]	11.1±0.3[a]
TP 80°C/3min	3.13±0.02[a]	0.82±0.01[a]	10.9±0.1[a]
TP 80°C/4min	3.13±0.01[a]	0.84±0.02[a]	11.0±0.4[a]
TP 80°C/5min	3.12±0.01[a]	0.83±0.01[a]	10.9±0.3[a]
TP 85°C/1min	3.13±0.02[a]	0.83±0.02[a]	10.9±0.2[a]
TP 85°C/2min	3.13±0.01[a]	0.83±0.01[a]	11.0±0.3[a]
TP 85°C/3min	3.14±0.01[a]	0.83±0.01[a]	10.9±0.3[a]
TP 85°C/4min	3.14±0.01[a]	0.83±0.01[a]	11.0±0.2[a]
TP 85°C/5min	3.13±0.02[a]	0.83±0.03[a]	11.0±0.1[a]
TP 90°C/1min	3.14±0.03[a]	0.83±0.02[a]	10.9±0.4[a]
TP 90°C/2min	3.13±0.01[a]	0.82±0.02[a]	10.9±0.3[a]
TP 90°C/3min	3.14±0.01[a]	0.83±0.02[a]	10.9±0.3[a]
TP 90°C/4min	3.14±0.02[a]	0.84±0.02[a]	10.9±0.3[a]
TP 90°C/5min	3.14±0.01[a]	0.83±0.03[a]	10.9±0.8[a]

Values are means and standard deviations of triplicate determinations. [a]Means with different letters in each columns are significantly different ($p < 0.05$).

[1] Expressed as percent citric acid g/100 g

Table 2.7 pH, titratable acidity and total soluble solids (°Brix) of Untreated and treated blueberry juice with continuous ultra-sonication flow rate and amplitude

Treatment	pH	Titrable acidity[1]	°Brix
Untreated	3.14±0.01[a]	0.84±0.01[a]	11.1±0.1[a]
US 24mL/min/40A	3.13±0.03[a]	0.83±0.01[a]	10.9±0.3[a]
US 24mL/min/80A	3.13±0.02[a]	0.83±0.02[a]	11.0±0.4[a]
US 24mL/min/100A	3.13±0.02[a]	0.83±0.02[a]	11.0±0.4[a]
US 93.5 mL/min/40A	3.12±0.01[a]	0.83±0.01[a]	10.9±0.3[a]
US 93.5 mL/min/80A	3.13±0.02[a]	0.84±0.01[a]	10.9±0.2[a]
US 93.5 mL/min/100A	3.12±0.01[a]	0.83±0.02[a]	10.9±0.6[a]

Values are means and standard deviations of triplicate determinations. [a]Means with different letters in each columns are significantly different ($p < 0.05$).

[1] Expressed as percent citric acid g/100 g (fresh weight basis)

In our study, the a* value for TP juice significantly decreased with increasing temperature and time. A similar pattern of reduction was observed by Aguilò-Aguayo et al., (2009b) in strawberry juice. They suggested the reduction of the a* value is mainly as a result of anthocyanin degradation and formation of Maillard reaction products. The reduction of the a* value in TP juices closely correlated with the anthocyanin degradation where red colour of the juice is exclusively due to the anthocyanins (Brunton and others 2009b).

As explained above, TP caused degradation of anthocyanins and juice colour while enhancing the undesirable reactions that lead to formation of HMF. However US treated juice (Table 2.9) does not exhibit significant changes in L* or in a* value, indicating the ability to preserve juice quality, which is in agreement with Perez et *al*. (2010).

Chroma corresponds to a color fading of juices. TP shows a significant reduction with increased temperature and time while US does not show a significant difference.

Table 2.8 Color parameters (L, a*, b*) of Untreated and treated blueberry juice with thermal pasteurization according to temperature and time

Treatment	L*	a*	Hue Angle	Chroma
Untreated	3.03±0.03^{a}	15.49±0.29^{a}	18.29±0.12^{a}	16.31±0.33^{a}
TP 80°C/1min	2.34±0.14^{b}	13.02±0.17^{b}	20.70±0.22^{a}	13.92±0.35^{b}
TP 80°C/2min	1.72±0.12^{c}	11.69±0.25^{c}	11.70±0.31^{g}	11.94±0.12^{c}
TP 80°C/3min	1.51±0.07^{d}	10.05±0.37^{ef}	12.61±0.67^{f}	10.30±0.35^{d}
TP 80°C/4min	1.42±0.05^{d}	9.68±0.28^{f}	14.20±0.55^{d}	9.99±0.25^{de}
TP 80°C/5min	1.36±0.10^{de}	8.90±1.05^{gh}	13.70±0.14^{e}	9.16±0.54^{ef}
TP 85°C/1min	1.98±0.07^{b}	11.31±0.13^{c}	14.24±0.35^{d}	11.67±0.43^{c}
TP 85°C/2min	1.62±0.11^{c}	11.04±0.74^{cd}	11.87±0.57^{fg}	11.28±0.32^{c}
TP 85°C/3min	1.46±0.08^{d}	9.83±0.23^{f}	14.48±0.29^{d}	10.15±0.68^{d}
TP 85°C/4min	1.33±0.09^{de}	9.23±0.25^{g}	16.41±0.22^{b}	9.62±0.73^{e}
TP 85°C/5min	1.28±0.06^{e}	8.70±0.32^{h}	15.85±0.18^{bc}	9.04±0.18^{ef}
TP 90°C/1min	1.74±0.11^{c}	11.31±0.30^{c}	14.38±0.46^{d}	11.68±0.41^{c}
TP 90°C/2min	1.56±0.08^{d}	10.46±0.33^{de}	11.98±0.35^{fg}	10.69±0.37^{cd}
TP 90°C/3min	1.40±0.04^{d}	9.76±0.18^{f}	13.09±0.53^{e}	10.02±0.31^{d}
TP 90°C/4min	1.23±0.09^{ef}	9.09±0.20^{g}	14.32±0.29^{d}	9.38±0.53^{ef}
TP 90°C/5min	1.11±0.10^{f}	8.55±0.22^{h}	15.11±0.68^{c}	8.86±0.51^{f}

Values are means and standard deviations of triplicate determinations. $^{a-h}$Means with different exponents in each columns are significantly different ($p \leq 0.05$).

Table 2.9 Color parameters (L*, a*, b*) of Untreated and treated blueberry juice vs. continuous ultra-sonication flow rate and amplitude

Treatment	L*	a*	Hue Angle	Chroma
Untreated	3.03±0.03[a]	15.49±0.39[a]	18.29±0.12[a]	16.31±0.33[a]
US 24mL/min/40A	2.89±0.63[a]	14.84±0.48[a]	14.69±0.71[b]	15.34±0.22[b]
US 24mL/min/80A	2.85±0.42[a]	14.79±0.86[a]	14.33±0.17[b]	15.27±0.37[b]
US 24mL/min/100A	2.83±0.23[a]	14.61±0.78[a]	12.20±0.34[c]	14.96±0.34[c]
US 93.5 mL/min/40A	2.91±0.43[a]	14.98±0.33[a]	14.66±0.33[b]	15.48±0.43[ab]
US 93.5 mL/min/80A	2.88±0.32[a]	14.76±0.85[a]	14.33±0.46[b]	15.23±0.82[b]
US 93.5 mL/min/100A	2.81±0.28[a]	14.62±0.98[a]	14.01±0.54[bc]	15.07±0.33[bc]

Values are means and standard deviations of triplicate determinations. [a-c]Means with different exponents in each columns are significantly different ($p \leq 0.05$).

2.3.6 Anthocyanin Degradation Kinetics

The thermal degradation of anthocyanin in blueberry juice was studied for the TP temperatures (80, 85 & 90 °C) used in the experiment. Figure 2.4, illustrates the degradation of blueberry anthocyanins as a function of heating time at each temperature.

The thermal degradation of blueberry anthocyanins follows first-order reaction kinetics with temperature, which was indicated by having correlation coefficient ($R^{2)}$ values of 0.9856, 0.9650 and 0.9430 for temperatures 80, 85 and 90 °C respectively (Table 2.10). Also the graph of C/C_O with time is linear (Figure 2.4). This observation is in accordance with the research work done by Guimarães et al. (2010) regarding degradation kinetics in blueberry juices using temperatures of 40, 50, 60, 70 and 80 °C. Similarly, degradation of anthocyanin follows a first-order reaction in juices such as sour cherry and strawberries (Brunton and others 2010a; Carle and others 2007).

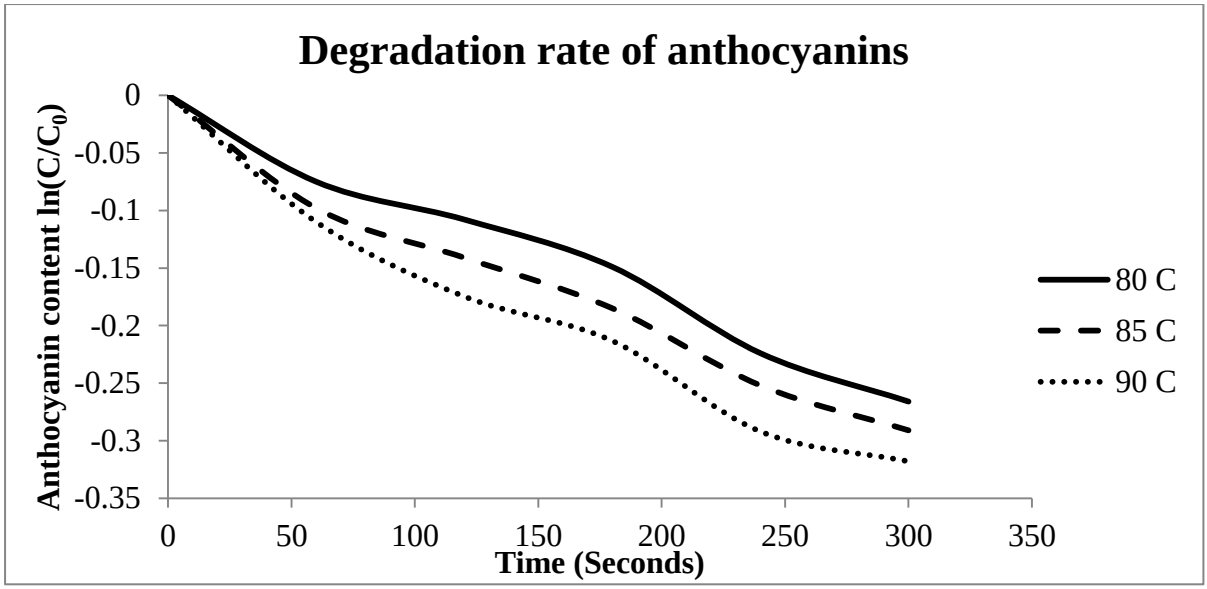


Figure 2.4 Degradation of anthocyanins in blueberry juice during heating at 80, 85 and 90 °C. Each point represents average of three replicates. Values are means of triplicate determinations

The anthocyanin degradation rate increases with increasing temperature with higher temperatures causing greater degradation (Hendrickx and others 2010). This can also be demonstrated by the rate constant (k) values obtained for different temperatures. The k value varied from 0.0009, 0.001, 0.0012 sec^{-1} at 80, 85 and 90 °C respectively (Table 2.10). The reported k values for these temperatures are much lower than previous research values. Guimarães et al. (2010) reported a k value of 0.0022 at 80°C. The $t_{1/2}$ value was 12.84, 11.55 and 9.63 h at 80, 85 and 90 °C temperatures respectively (Table 2.10). These values are much higher than the previous research findings. Guimarães et al. (2010) indicated the $t_{1/2}$ value of blueberry juice at 80°C as 5.11 hr, and they did not study the kinetics beyond 80 °C. The high $t_{1/2}$ value indicates greater stability of anthocyanins in different heating temperatures where our results show a reduced stability with increasing temperature from 80 to 90 °C. Some researchers argue that the difference in $t_{1/2}$ value of anthocyanins present in different types of juices will be a result of different anthocyanin forms and possible interaction of anthocyanin with other fruit constituents (Acosta and others 2009). According to previous research work the anthocyanin stability in blueberry juice is higher than that in the juices from blackberry

due to greater $t_{1/2}$ at the same temperature (Guimarães and others 2010; Buckow and others 2010).

Table 2.10 Effect of temperature on k and $t_{1/2}$ values of anthocyanin degradation in blueberry juice

Temperature (°C)	k × 10^3 (sec-1)	$t_{1/2}$ (h)	R^2
80	0.9±0.03	12.84±0.88	0.9856
85	1.0±0.01	11.55±0.54	0.9650
90	1.2±0.01	9.63±0.21	0.9430

k – degradation rate constant; $t_{1/2}$ -- half-life; R^2- square of correlation coefficient. Values are means and standard deviations of triplicate determinations

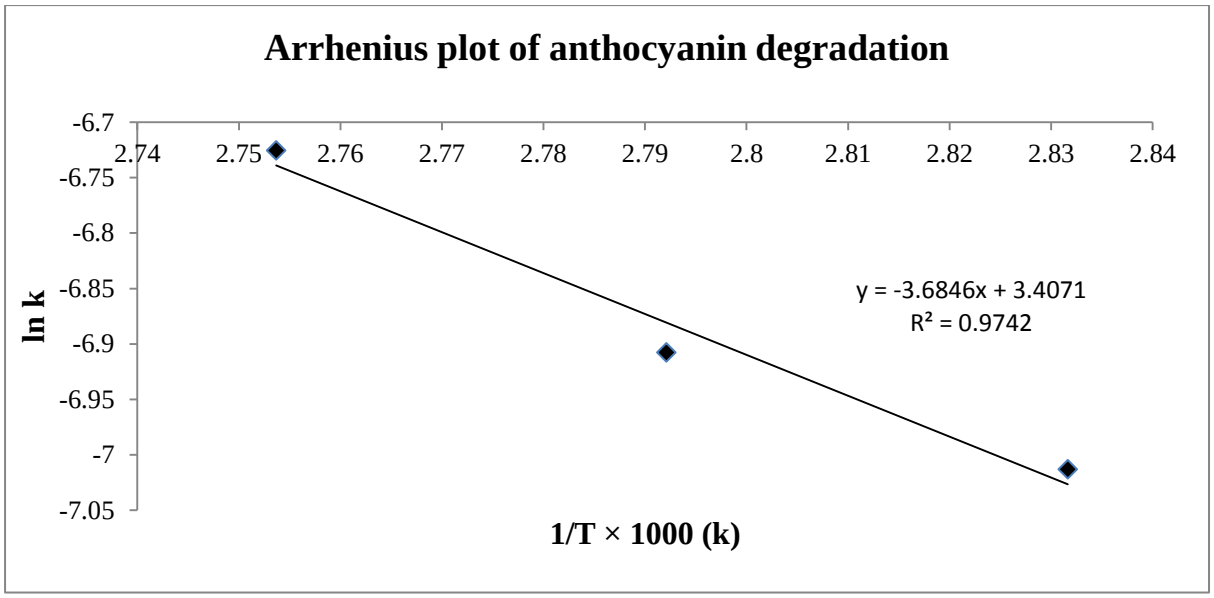


Figure 2.5. The Arrhenius plot for degradation of anthocyanin pigments in blueberry juice. Values are means of triplicate determinations

The temperature dependence of the rate constants is shown using the Arrhenius plot (Figure 2.5). According to our results (Table 2.11), the activation energy of blueberry anthocyanins is 30.64 $kJmol^{-1}$. This value is much lower than the value of 80.42 determined by Guimarães et al., (2010). Higher activation energy indicates that a smaller temperature is required in order to degrade a specific compound (Prapulla and others 2005).

Table 2.11 Activation energy of anthocyanin degradation

Temperature (°C)	T (Kelvin)	k	Activation energy ($kJmol^{-1}$)
80	353.15	0.0009	30.64
85	358.15	0.001	
90	363.15	0.0012	

k – degradation rate constant; T – absolute temperature. Values are means of triplicate determinations

The Q_{10} measures degradation rate of anthocyanin as a consequence of increasing the temperature by 10 °C. The temperature coefficient (Q_{10}) of blueberry anthocyanin degradation for the temperature range of 80 to 90 °C is 1.333±0.02. This value follows the same pattern of Q_{10} obtained in blueberry juice studies conducted by Guimarães et al., (2010). They observed a reduction of Q_{10} value in juices with increasing temperature from 40 to 80 °C. They reported a value of 2.95 at 60 to 70 °C and a value of 1.67 at 70 to 80 °C. In their study, the high Q_{10} value related to the greater dependence of anthocyanin degradation is occurred at the temperature range of 60 to 70 °C. In present study, low Q_{10} value at the temperature range of 80 to 90 °C shows a lower influence of temperature at this range on anthocyanin degradation kinetics.

2.4. Conclusions

The present study demonstrated that US processing preserves the anthocyanins and juice color better than conventional TP while still providing significant reduction in microbial counts of APC, TC, yeast and mold. Thus US has the potential, with further development, to be an alternative to thermal pasteurization and which would result in a higher quality juice. The continous flow method of US method used in this study allows continous juice processing (as does commercial thermal pasteurization equipment), which should be useful for commercial implementation. However, optimum US conditions of flow rate and amplitude must be estabilished in order to provide a 5-log reduction in pertinent microorganisms present in the juices. Combining other antimicrobial methods might provide

more inactivation than by US alone. The degradation kinetic studies of anthocyanin with temperature allows prediction of possible of juice quality loss during TP.

CHAPTER 3 EFFECTS OF BLUEBERRY (*VACCINIUM CORYMBOSUM*) JUICE ON LIPID OXIDATION DURING SPRAY DRYING OF MICROENCAPSULATED SALMON OIL AND MENHADEN OIL

3.1 Introduction

Microencapsulation is defined as a process by which small particles of core material such as fish oil and flavors are enveloped within a wall material to form micro size capsules (Assadpoor and others 2008a; Drusch and Schwarz 2006b; Huang and Klaypradit 2008). Although many methods are available for microencapsulation, spray drying is the most common technique employed to produce micro capsules food related applications because equipment is readily available for producing good quality micro-capsule powders (Assadpoor and others 2008b). Also, spray drying is known to be an economical (Ersus and Yurdagel 2007; Drusch and Mannino 2009b) and flexible operation. The spray drying microencapsulation process involves conversion of liquid oil or flavors in the form of an emulsion into a dry powder, protecting the encapsulated material from further oxidation. Spray drying is a one-step continuous operation of drying involving several stages such as feed atomization, mixing of hot air and feed spray, evaporation and separation of produced powders (Langrish 2009).

Fish oils have recently received greater attention because of the presence of omega-3 type long chain polyunsaturated fatty acids (PUFA). Polyunsaturated fatty acids especially omega 3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to have preventive and curative effects on cardiovascular disease, cancers and impediments to infant neural development (Law and others 2001; Kruif and others 2001; Jaworska and others 2007; Bralley and others 2009; Gilmore and others 2000). Menhaden (*Brevoortia patronus* and *Brevoortia tyrannus*) oil has been reported to have an EPA and DHA content of 12.8 – 15.4% and 6.0 – 9.1% respectively (IFFO 2005) whereas salmon

(*Oncorhynchus nerka*) oil levels were 13% and 18% (Beindroff and Zidam, 2010) respectively. According to previous research findings (Behchtel and Wu 2008; Garg and others 2006), decreased rate of cardiovascular incidences were reported in populations with greater fish consumption. Most western diets are rich in saturated fats compared to unsaturated fatty acids (Laufenberg and Kolanowski 2006). The American Heart Association recommends intake of EPA and DHA for coronary heart disease patients and people having high triglyceride levels, at levels of 1g per day and 2 -4 g/day respectively (Appel and others 2002; Browning and others 2006). Instead of changing the dietary pattern of these individuals, which is difficult to do in practice, increased intake of fish oil can be achieved by food enrichment with fish oil. But direct incorporation of fish oil into food is associated with problems such as reduced sensory acceptance and reduced product shelf life. The PUFA are highly unstable and undergo quick oxidation resulting in formation of toxic hydro-peroxides and objectionable off flavors (Kolanowski and others 2006). Microencapsulation of fish oil is a technique developed to overcome these constraints. Successful fish oil microencapsulation results in the prevention of contact of the oil with oxygen or metal ions, direct light exposure and the trapping off flavors (Beindroff and Zidam 2010; Auty and others 2001; Bohin and others 2006). Although spray drying is the most popular microencapsulation technique used in the food industries, it is known to induce lipid oxidation and produce powders with porous structure (Liu and Yang 2011; Huang and Klaypradit 2008). Though low spray drying temperatures are advantageous, fish oil microencapsulation requires temperatures as high as 170°C (Drusch and others 2006c; Chambin and others 2007) which might induce lipid oxidation during the process of spray drying (Drusch and others 2009c). Beindroff and Zidam (2010) suggested the use of nitrogen gas while spray drying and producing powders using two step processes where the particles are initially half dried using a spray drier and further dried using a filtermat spray dryer or fluidized bed dryer (Bhandari and Jafari 2007a).

Although these methods reduced lipid oxidation, they significantly increased the cost of production. The addition of antioxidants such as tocopherol or trolox C into the fish oil emulsions has been suggested for increasing the storage stability of fish oil. Even though these antioxidants can act as pro-oxidants in higher concentrations, it was found that combination of antioxidants such as tocopherol, ascorbyl palmitate and rosemary extract significantly retarded the auto-oxidation of fish oil microcapsules upon storage (Baik and others 2004). The addition of synthetic antioxidants into fish oil microcapsules may provide a negative perception among health conscious consumers. The research related to incorporation of natural antioxidants into fish oil emulsions is scant. Natural antioxidants might reduce the possible lipid oxidation occurring during the process of emulsification (ultra-sonication) and spray drying, and provide greater storage stability in microencapsulated powders (even with a high load of core material) and still retain a positive perception among consumers.

Berries, especially blueberries (*Vaccinium corymbosum*) are considered to be a good source of phenolic compounds. These fruits have received much attention owing to their high antioxidant activity as compared to many other fruits and vegetables. Polyphenols and anthocyanins present in blueberries are known to have a positive role in human health and disease prevention (Brownmiller and others 2008; Wang and Zheng 2003). The berry antioxidants can scavenge free radicals in oils, and also have the ability to chelate pro-oxidants such as metal ions. This could result in reduced lipid oxidation and increased fish oil stability during emulsification, spray drying, and subsequent storage. The objectives of this study were to: (1) determine the effect of blueberry juice addition on lipid oxidation during emulsification and spray drying of microencapsulated menhaden and microencapsulated salmon oil; (2) evaluate their emulsion and powder characteristics; and (3) estimate their microencapsulated powder production rate and energy efficiency.

3.2 Materials and Methods

3.2.1 Preparation of Blueberry Juice (BJ)

Three batches of fresh imported high bush blueberries (*Vaccinium corymbosum* L.) obtained locally were steam blanched using a steamer for 2 min and rapidly cool down to 10 °C using an ice water bath. Berries were then blended using a mechanical blender (Magic bullet, China) for 1 min and the mash was centrifuged at 2037.6×g using a centrifuge (J2-HC-TB-002 model, Beckman instruments Inc., California, USA) for 20 min at 4 °C to separate the liquid phase from the solid phase.

3.2.2 Physico Chemical Properties of BJ

Blueberry juice yield was calculated as: (weight of juice/initial berry weight)*100. Total monomeric anthocyanin content (TAC) of the blueberry juice was determined by the pH differential method of AOAC 2005.02 (AOAC, 2005b) as described in section 2.2.6. The results were expressed as mg of cyanidin-3-glucoside equivalents of per liter of juice.

Total phenol content (TPC) of blueberry juice was determined according to the Folin-ciocalteau method (Sinkard and Singleton 1997) as described in section 2.2.7. The results are expressed as milligrams of gallic acid equivalent (GAE) per liter of juice.

The antioxidant activity (AA) of blueberry juice was determined by employing the DPPH radical scavenging assay (1,1-Diphenyl-2-picryl-hydrazyl) according to the method of Arlorio et al. (2009) with some modifications. More details of the analyses were provided in 2.2.8. The results were expressed as µmol of trolox equivalent (TE) per 100 mL of juice.

Juice pH value was measured using a bench top pH meter (Symphony, VWR Scientific 5B70P, PA, USA). The titratable acidity was determined using a titration method as described in section 2.2.9, and results were expressed as percent total organic acid on the basis of citric acid which is the predominant acid present in blueberries (Ehlenfeldt et. al.

2004). °Brix of the juice was measured at 20 °C using a hand held digital refractometer (Model AR 200, Reichart Analytical Instruments, USA).

Color of the juice was measured using a Lab Scan XE Colorimeter (Hunter Associates Laboratory, INC. Resbon, VA) and was reported in CIE LAB color scales (*L**, *a** and *b** values).

3.2.3. Physico-chemical Properties of Fish Oil

Purified menhaden (*Brevoortia patronus)* oil (without added antioxidant) was provided by OmegaPure^{TM}, (Omega Protein,Inc, Houston, TX). Crude salmon (*Oncorhynchus nerka*) oil was donated by a commercial fish meal processor, Kodiak, AK. The unpurified salmon oil was mixed with 5% of activated earth and stirred for one hour at room temperature using a magnetic stirrer. After that the mixture was centrifuged (J2-HC-TB-002 model, Beckman instruments Inc., California, USA) at 12000×g for 25 min at 4 °C and the purified salmon oil was separated and used for this study.

Both menhaden oil (MO) and salmon oil (SO) were analyzed for peroxide value (PV) according to the AOCS official method cd 8-53 (1997). Five (g) of fish oil samples were placed in the Erlenmeyer flask and dissolved using 30 mL of acetic acid and chloroform solution (Sigma-Aldrich co., St Louis, MO, USA) (3:2) and then 0.5 mL of saturated potassium iodide solution (Sigma-Aldrich co., St Louis, MO, USA) was added. The mixture was stirred for a few seconds. After 1 min, 30 mL of deionized water was added and titrated with 0.01 N sodium thiosulfate solution (Sigma-Aldrich co., St Louis, Mo, USA) using starch as the indicator. PV was calculated using Eq (3.1) and reported as milliequivalents of peroxide per 1 kg of oil using following formula (3.1):

$$PV = \frac{(S - B) \times N \times 1000}{m} \qquad (3.1)$$

Where B is the volume of titrant (mL) of blank; S is volume of titrant (mL) of sample; N is normality of sodium thiosulfate solution, and m is mass (Kg) of the oil sample.

p-andisine value (p-AV) which represents secondary lipid oxidation products in the oils was estimated using the method AOCS official method cd 18-90 (1997). One gram (g) of fish oil samples were placed in a 25 mL volumetric flasks and dissolved with isooctane (2,2,4-trimethylpentane) obtained from Sigma-Aldrich Co., St Louis, MO, USA. The absorbance (Ab) of these solutions was measured at 350 nm using a spectrophotometer (Thermo Fisher Scientific, Vernon Hills, IL, USA). Then 5 mL of this fish oil solution was transferred into a test tube and 1 mL of p-anisidine (Sigma-Aldrich co., St Louis, Mo, USA) reagent (0.25 g/100 mL) was added. After 10 min, the absorbance (As) of this mixture was measured at 350 nm using a reference blank. The p-AV was calculated using the formula (3.2).

$$\text{p-AV} = \frac{25 \times (1.2As - Ab)}{m} \qquad (3.2)$$

The Totox values of the oils were calculated using the equation: Totox = AV + 2PV as described by AOCS (cg 3-91, 2007). Totox provides the overall oxidation state of the oils.

The color of the fish oils were measured using a Lab Scan XE Colorimeter (Hunter Associates Laboratory, INC. Resbon, VA) and was reported in CIE LAB color scales (L^*, a^* and b^* values). Chroma and hue angle value were calculated using the Equations (3.3) and (3.4), respectively. The negative hue angle values were converted into positive values by adding 180°, in order to make them fall in the 90° – 180° quadrant (+b* = yellow; -a* = green).

$$\text{Chroma} = [a^{*2} + b^{*2}]^{1/2} \qquad (3.3)$$

Hue angle = $\tan^{-1}$ (b*/a*) (3.4)

3.2.4 Preparation of Feed Emulsions

Oil in water emulsions were prepared with MO or SO containing 5% or 10% of BJ. The emulsions containing MO (EMO), MO with 5% BJ (EMOBJ) and MO with 10% BJ (10EMOBJ), and SO containing SO (ESO), SO with 5% BJ (ESOBJ) and SO with 10% BJ (10ESOBJ) was prepared.

All of the emulsions were prepared using OSA modified starch and corn syrup of dextrose equivalent of 36/43 (ADM company, Decateur, IL) as wall materials. The emulsions were prepared according to the formulation shown in Table 3.1 based on preliminary experiments related to emulsion stability.

Table 3.1. Emulsion formulation (% w/w) to produce microencapsulated MO & SO powders

	% Wall material		% Core material			
Emulsions	OSA starch	Corn syrup (DE 36/43)	MO	SO	% BJ	% Demineralized water
EMO	5	10	20	-	0	65
5EMOBJ	5	10	20	-	5	60
10EMOBJ	5	10	20	-	10	55
ESO	6	10	-	20	0	64
5ESOBJ	6	10	-	20	5	59
10ESOBJ	6	10	-	20	10	54

Wall materials were dissolved in water and then BJ was added to the solutions. The oils were separately added to the mixture of the wall material, and stirred before the emulsions were further emulsification using an ultrasonic processor (Model CPX 500, Cole Palmer Instruments, Vernon Hills, Il, USA) having a 20 KHz probe. Emulsification was done at the pulse rate of 2 to 1 for 5 min. The amplitude level was set to 80% for sonicating the

emulsions. During the emulsification process the Temperature of sample containers were kept at 20 ^{o}C using an ice bath.

3.2.5 Emulsion Stability, pH and Color

The stability of the emulsions was evaluated according to a method described by Min and others (2003) with modifications. Five grams of an emulsion sample was placed in a 10 mL centrifuge tube, and stored at -20 °C for 2 days. After that the sample was allowed to thaw at room temperature for one hour. Then the sample was centrifuged at 15000×g for 40 min at -2 °C, and the amount of oil separated was measured. The oil recovery percentage was calculated using formula 3.5. The emulsion stability was determined using the formula 3.6.

$$\text{Oil recovery percentage} = \frac{\text{weight of oil recovered}}{\text{Emulsion weight (5g)}} \times 100 \ \% \quad (3.5)$$

$$\text{Emulsion stability} = 100\% - \text{Oil recovery percentage} \quad (3.6)$$

Emulsion pH value was measured using a bench top pH meter (Symphony, VWR Scientific 5B70P, PA, USA). Color of the emulsions were measured using a Lab Scan XE Colorimeter (Hunter Associates Laboratory, INC. Resbon, VA) and was reported in CIE LAB color scales (L*, a* and b* values).

3.2.6 Emulsion Rheological Properties

Emulsion flow properties and viscoelastic properties were determined using an AR 2000ex Rheometer (TA Instruments, New Castle, DE). The Rheometer was fitted with a plate geometry employing 40 mm diameter and 400 µm sample gap. The emulsion sample was placed on the temperature controlled plate and allowed to equilibrate at temperatures of 5, 15 and/or 25 ºC. The shear stress of the emulsions was measured at shear rates of 1 to 200 s^{-1}. The emulsion flow properties were analyzed using the power law (Equation 3.7) model where

σ represents shear stress (Pa); γ symbolizes shear rate (s^{-1}); K represent consistency index ($Pa.s^n$), and n symbolize flow behavior index.

$$\sigma = k\,\gamma^n \tag{3.7}$$

A linear plot of log σ and log γ was constructed, and the magnitude of k and n were determined from the intercept and slope respectively. The k, n and apparent viscosity of the emulsions were reported. A plot was constructed for each temperature of shear viscosity against shear rate.

The viscoelastic properties of emulsions were determined by conducting a frequency sweep test using a frequency range of 0.1 to 10 Hz, and a constant temperature of 25 °C. The storage (G`) and loss (G``) modulus (Pa) curves against angular frequency were obtained from Universal Analysis (TA instrument) software, and they were calculated according to the following Equations 3.8 and 3.9. The lost tangent value was calculated (Equation 3.10) using G` and G``. A plot was constructed of δ vs. angular frequency.

$$G` = \frac{\sigma_0}{\gamma_0} Cos\delta \tag{3.8}$$

$$G`` = \frac{\sigma_0}{\gamma_0} Sin\delta \tag{3.9}$$

$$Tan\delta = \frac{G``}{G`} \tag{3.10}$$

Where tan δ represents the lost tangent, σ represents generated stress and γ represents oscillating strain.

3.2.7 Preparation of Spray Dried Microcapsules

The infeed emulsions were transformed into microcapsules using a pilot plant spray drier (FT 80 Tall Form Spray Dryer, Armfiled Inc., Jackson, NJ, USA) at the Food Processing Pilot Plant, Louisiana State University Agricultural Center following a co-current drying condition (Figure 3.1). The spray drier is composed of a cylindrical chamber of 1.7 m

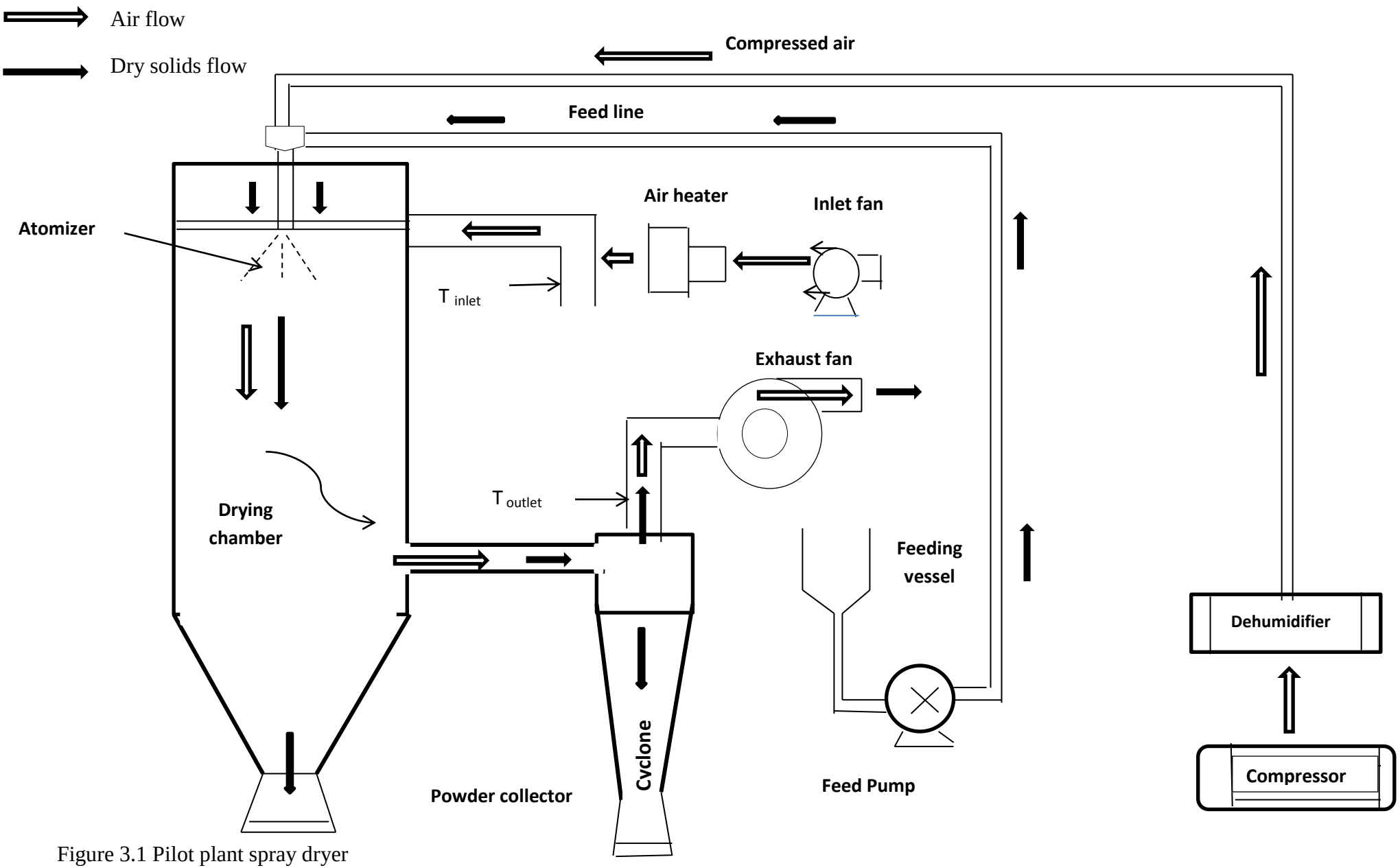


Figure 3.1 Pilot plant spray dryer

height and 0.3 m diameter, and equipped with a pressurized air atomizing nozzle and automated introduction of the feed into the drying chamber through a feed pump. The operational conditions of the spray drying were: inlet air temperature 170 °C, and nozzle air gauge pressure of 0.7 bars. The emulsions of EMO, 5EMOBJ and 10EMOBEJ were spray dried to produce microencapsulated powders of MMO, 5MMOBJ and 10MMOBJ respectively. Similarly emulsions of ESO, 5ESOBJ and 10ESOBJ were spray dried to produce powders of MSO, 5MSOBJ and 10MSOBJ respectively. The dried powders were collected and stored in amber bottles at 4°C for further analysis.

3.2.8 Lipid Oxidation Parameters of Feed Emulsions and Microcapsules

The microencapsulated oil from emulsions was extracted out according to a method described by Shahidi and others (2007). Ten grams of emulsion were mixed with 100 mL of chloroform (Sigma-Aldrich co., St Louis, MO, USA) 50 mL of methanol (Sigma-Aldrich co., St Louis, MO, USA), and a few crystals of tert-butylhydroquinone (TBHQ). The mixture was centrifuged (J2-HC-TB-002 model, Beckman instruments Inc., CA, USA) at 12000×g at 4 ^{o}C for 5 min. The centrifuged mixture was transferred into a separatory funnel, and the chloroform layer was removed. Oil was recovered by evaporating the chloroform layer using a rotary evaporator (Rotavapor, Model 121, Bűchi Co., Switzerland) under vacuum at 40 °C.

The same technique was used to extract oil from microcapsules with minor modifications. Ten grams of microencapsulated powders were dissolved in 50 mL of 0.88% (w/w) KCl (Sigma-Aldrich co., St Louis, Mo, USA) solution. Then 100 mL of chloroform, 50 mL of methanol, and a few crystals of tert-butylhydroquinone (Sigma-Aldrich co., St Louis, MO, USA) were added into the mixture. The mixture was centrifuged as previously described and the oil was recovered from the solvent at 40 °C.

The recovered oil from emulsions and microcapsules were analyzed for PV (AOCS cd 8-53, 1997), p-AV (AOCS cd 18-90, 1997), Totox (AOCS cg 3-91, 1997) as described in section 3.2.3.

3.2.9 Fatty Acid Methyl Ester (FAME) Profile Analysis

Fatty acid methyl esters (FAME) of MO, SO, and oil extracted from the microcapsules were prepared according to the method described by Maxwell and Marmer (1983). Approximately 20 mg of oil sample was mixed with 4.5 mL of isooctane, 500 µl of internal standard (10 mg methyl tricosanoate (23:0)/mL isooctane) and 500 µl of 2N KOH (1.12g/10 mL methanol) in a glass test tube. The contents were vortexed for 60 s and centrifuged for 3 min at 3200 rpm. The lower methanol layer was discarded. One mL of saturated ammonium acetate solution was added into the tube and stirred with the vortex. The mixture was centrifuged and the aqueous layer was removed and discarded. The above procedure was repeated with 1 mL of water instead of ammonium acetate. Two to three g of anhydrous sodium sulfate was placed in the tube and the mixture was vortexed and then allowed to stand for 30 min. Then the mixture was centrifuged for 15 min at 3200 rpm. The upper isooctane layer (0.5 mL) was transferred into an amber GC vial. 0.5 mL of isooctane added and contents were stored at -70 °C for further analysis.

The fatty acid methyl esters were analyzed using a gas chromatograph (model 7890A Agilent) coupled with a flame ionization detector (Agilent Technologies Inc, Santa Clara CA., USA). The separation of the methyl esters was conducted using a FAMEWAX^{TM} (RESTEK, USA) capillary column (30m × 0.32 mm i.d. × 0.25 µm, Restek, Bellefonte, PA). Data was collected and analyzed with the GC ChemStation program (ver E.02.00.493 Agilent Technologies, Inc.). Helium gas was used at an average velocity of 64cm/sec as a carrier gas. The injector and detector temperatures were set to 250 °C and 280 ºC respectively. A split

injection was used (50:1 split ratio), and the injection volume was 1 µl. The following oven program was employed: 195 ºC to 240 ºC at a rate of 5 ºC/min for 9 min and holding time of 2 min for a total run time of 11 min. The peaks were identified using retention times of standards: Supelco 37, PUFA #1, PUFA #3, and cod liver oil from Supelco (Bellefonte, PA). Data was expressed as percent of total integrated area (Oliveria and Bechtel 2005).

3.2.10 Microencapsulation Efficiency Analysis

Encapsulation efficiency designates the amount of core material encapsulated within the powder particles. The microencapsulation efficiency (ME) of spray dried powders were calculated using surface oil content and total oil content according to 3.11.

$$ME = \frac{\text{Total oil content} - \text{Surface oil content}}{\text{Total oil content}} \times 100 \qquad (3.11)$$

Surface oil content refers to the free or extractable oil around microcapsules. The surface oil contents of microencapsulated fish oil powders were determined using petroleum ether as the extraction solvent using a method described by Assadpoor and others (2008a) with minor modifications. Ten gram of microencapsulated powder was added to 100 mL of petroleum ether (Sigma-Aldrich co., St Louis, MO, USA), and the resultant suspension was manually shaken. Then the suspension was placed on an automatic shaker (Lab-Line 3525 incubator shaker, International MI-SS Inc. Corona, CA) for 15 min and then the mixture was filtered using a Whatman 4 filter paper (Whatman International Ltd, England). The powder residue was washed using 5 mL of the solvent three times. The filtrate solution and washed solvent was transferred into a round bottom flask and the solvent was evaporated at 60 ºC using a rotary evaporator (Rotavapor, Model 121, Bűchi Co., Switzerland) under vacuum at 4 ºC to recover the oil. After evaporation, the flask was dried in a hot air oven (VWR International, PA, USA) at 103 ºC for 1 h. The surface oil content was measured based on the

weight difference between the initial clean flask and the flask with the extracted oil residue. The results were expressed as amount of surface oil content per gram of microcapsules.

The total oil content of the microcapsules which include both encapsulated and surface oil, was determined as described by Augustin and others (2009) with some modifications. The microcapsules (0.5 g) were initially digested with 1 mL of 36% w/w hydrochloric acid (Sigma-Aldrich co., St Louis, MO, USA) in a screw cap test tube at 100 ºC for 10 min. The tube was cooled to room temperature and 10 mL of ethanol (96% v/v) (Sigma-Aldrich co., St Louis, MO, USA) was added. Twenty filve mL of Petroleum ether (Sigma-Aldrich co., St Louis, MO, USA) and twenty five mL of diethyl ether (1:1) (Sigma-Aldrich co., St Louis, Mo, USA) was used to extract the oil from the mixture. The extracted solvent was removed using a rotary evaporator (Rotavapor, Model 121, Bűchi Co., Switzerland) and the flask with the residue was dried using a hot air oven at 100 ºC for 1 h. The total oil content was calculated by gravimetric difference and expressed as the oil content per gram of microcapsules.

3.2.11 Color of Microencapsulated Fish Oil Powder

The color of the microcapsules was measured using a Lab Scan XE Colorimeter (Hunter Associates Laboratory, INC. Resbon, VA) and was reported in CIE LAB color scales (*L**, *a** and *b** values).

3.2.12 Morphology of Microcapsules

The morphology of microcapsules was observed using a scanning electron microscope (SEM) using an acceleration voltage of 5 kV. Microcapsules were mounted onto an aluminum SEM specimen stub using a double sided sticky tape, and coated with gold:palladium (60:40) using S150 sputter coater (Edwards High Vacuum International,

Wilmington, MA) to make the samples conductive. The coated stubs were examined and imaged with SEM.

3.2.13 Particle Size Distribution Analysis

The particle size of the microcapsules was measured using a Microtrac S3500 light scattering system (MicroTrac, Largo FL, USA). The system consists of three fixed 780 nm solid state lenses with a computerized single lens alignment. The measurement capacity was from 0.24 to 2800 mm diameter. Initially a certain amount of powder sample was placed in the test chamber with circulating ethyl alcohol (Sigma-Aldrich co., St Louis, MO, USA). Before each test a 10 s period of ultrasound mixing was conducted at 20 W. The sample was pumped through the sample cell at 40% of the maximum flow rate. Light was scattered from the tri lasers from low to high angles (0-163°), and the complete light scattering pattern was collected. Modified Mie-scattering technique was employed to calculate the volume distribution of particle size using light scattering pattern. Results were noted as 50^{th} (median) and 90^{th} percentiles of the microcapsules sizes.

3.2.15 Statistical Analysis

Mean values and standard deviations of triplicate experiments were reported. Analysis of variance (ANOVA) was carried out to determine the difference among treatments means (SAS Version 8.2, SAS Institute Inc., Cary, NC) using the post hoc Tukey's Studentized Range Test.

3.3. Results and Discussion

3.3.1 Physico-chemical Properties and Percent Yield of BJ

Blueberry juice yield was 61% (w/w) of liquid matter separated from the residue. Blueberry seeds, mucilage and pectin were contained in the residue. BJ contained 106.38 of TAC (mg/L cya-3-glu) and 3273 of TPC (mg/L gallic acid equivalents) (Table 3.2). The BJ

had high a* and low L* values, which indicated the juice was dark reddish purple in color. The BJ was acidic in nature having a pH value of 3.23 and titratable acidity of 0.83 (citric acid equivalents).

Table 3.2 Physico-chemical properties and yield (%) of BJ

BJ yield (%)		61.04±3.93
TAC [a]		106.38±9.54
TPC [b]		3273±22.31
AA [c]		3021.70±232.22
^{0}Brix[e]		11.3 ±1.76
pH[e]		3.23±0.34
Percent Titratable acidity [d]		0.83 ±0.03
Color	L*	6.65±0.03
	a*	32.11±0.04
	b*	11.32±0.05

[a] Expressed in mg/l of cyanindin-3-glucoside equivalents.

[b] Expressed in mg/l gallic acid equivalents.

[c]Expressed in µmol/100 mL Trolox equivalents.

[d]Expressed based on ctiric acid.

[e]The values are taken at 20 °C.

Values are means and SD of triplicate determinations. BJ=blueberry juice; TAC= total anthocyanin content; TPC = total phenol content; AA=antioxidant activity.

Blueberries showed high antioxidant activity due to the presence of anthocyanins and polyphenols. Blueberries contain myricetin, quercetin, chlorogenic acid and kaempferol as phenolic compounds, where chlorogenic acid contributes mostly to the antioxidant activity. It has been reported that Anthocyanin contributes equally as phenolic acid related to antioxidant activity of blueberries (Wang and Zheng 2003).

According to the US Department of Agriculture, blueberries ranked as number one in antioxidant activity based on ORAC assay (oxygen radical absorbing capacity) compared to 40 common fresh fruits and vegetables. Fresh blueberries contain 2400 ORAC units/100 grams (Booth and others 1998; Cao and others 1998), which is higher than other berries including grapes, strawberries, raspberries and cranberries. Several methods are available to determine antioxidant activity, where in this study DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to measure the antioxidant activity of BJ. The DPPH mainly addresses the radial scavenging ability of an antioxidant (Faria and others 2005) which is appropriate to indicate antioxidant activity in a lipid system, where lipid oxidation is carried out by a series of free radical reactions. The value obtained (3022 µmol TE/100g) (Table 3.2) was lower than the values reported in the previous study related to blueberry fruit based on DPPH assay. Bi et al. (2010) reported a total antioxidant activity (5630-7600 µmol TE/100g) based on DPPH assay in many blueberry cultivars.

Antioxidant compounds, such as phenolic acids, polyphenols and flavonoids, present in the berries are able to scavenge free radicals as peroxide, hydro-peroxide and lipid peroxide, and thus inhibit oxidative mechanisms that lead to lipid oxidation. Often, for ease of comparison antioxidant activity of these compounds are represented based on a common reference standard such as trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Many previous research studies represent the total antioxidant activity of different fruits and vegetables by combining lipophilic and hydrophilic antioxidant values. Frankel (1996) has reported that lipophilic antioxidant including tocopherol and ascorbyl palmitate are more effective in oil-in-water emulsions than in bulk oil compared to hydrophilic antioxidants (trolox and ascorbic acid). Blueberries are found to have both hydrophilic and lipophilic antioxidants which make them able to work effectively in both oil in water emulsions and bulk oil (Beecher and others 2004).

3.3.2 Lipid Oxidation Parameters and Color of Purified MO and SO

Table 3.3 shows the physico-chemical characteristics of menhaden and salmon oil. According to Table 3.3, menhaden oil and salmon oil had PV of 2.9 meq/kg and 2.73 meq/kg, respectively. p-AV value for menhaden oil and salmon oil had 5.64 and 9.3, respectively.

Table 3.3 PV, AV, Totox and color of MO and SO

		MO	SO
PV[x]		2.90 ± 0.15	2.73±0.59
p-AV		5.64±0.36	9.30±2.13
Totox value		11.44±0.79	14.76±2.76
Color	L*	77.93±0.02	67.05±2.45
	a*	7.57±0.01	5.87±3.09
	b*	18.54±0.01	55.68±1.79

[x]meq peroxide /kg oil

Values are means and SD of triplicate determinations. MO= menhaden oil; SO = salmon oil; PV=peroxide value; AV=anisidine value.

Lipid oxidation results from chain reactions, where oil oxidizes and produces primary oxidation products such as peroxides, dienes and free fatty acids, which lead to secondary oxidation products such as carbonyls, aldehydes and trienes. Marine oils are highly susceptible to lipid oxidation due to the large amount of polyunsaturated fatty acids in the oil (Drusch and others 2010). Peroxide value (PV) measures the primary oxidation product of hydroperoxides, while p-anisidine measures secondary oxidation products. The Totox value indicates the overall rate of lipid oxidation. According to quality standards published from a trade association located in Washington USA i.e. council for responsible nutrition (CRN) and Norwegian company EPAX, a world leading supplier of marine derived omega-3 fatty acid, the good quality marine oil should have peroxide value of less than 5 meg/kg (Kuley and

others 2009) and p-anisidine value less than 20. The oils used for the present study were below than the suggested cut offs, which indicated oil used for the experiment was of good quality initially.

3.3.3 Emulsion Stability, pH and Color of Emulsions

All of the prepared menhaden and salmon oil emulsions (with or without the addition of blueberry juice) had high emulsions stability (Table 3.4 and 3.5). Emulsions with blueberry added had significantly increased stability over EMO and ESO. This may be due to the lipophilic and hydrophilic compounds present in the juice which might aid in further emulsion stabilization.

Table 3.4 Emulsion Stability, pH and Color of MO Emulsions

		EMO	5EMOBJ	10EMOBJ
% Emulsion stability		98.76±0.36^{b}	99.93±0.09^{a}	99.64±0.33^{a}
pH		4.93±0.02^{a}	4.55±0.03^{b}	4.32±0.02^{c}
color	L	88.42±0.62^{a}	84.92±0.24^{b}	81.63±0.18^{c}
	a*	-2.80±0.08^{c}	1.07±0.02^{b}	2.68±0.19^{a}
	b*	2.74±0.04^{a}	0.76±0.17^{b}	0.53±0.05^{c}
hue angle		139.55±0.14^{a}	34.09±0.57^{b}	19.57±0.97^{c}
Chroma		3.78±0.10^{a}	1.52±0.02^{c}	3.36±0.18^{b}

Values are means and SD of triplicate determinations. $^{a-c}$Means with different exponents in the each row indicate significant difference ($p \leq 0.05$).
MO=menhaden oil; EMO= menhaden oil emulsion; 5EMOJ=menhaden oil emulsion with 5% blueberry juice; 10EMOBJ=menhaden oil emulsion with 10% blueberry juice.

However salmon oil emulsions have less stability than menhaden emulsions because of having impurities in the salmon oil. A stable emulsion with well dispersed fine droplets of the core material is critical for microencapsulation (Fujishima and others 2003). The microencapsulation efficiency was expected to be affected by emulsion stability where higher

stability interconnected with higher efficiency (Augustin and others 2006). The pH value of the emulsions decreased with the juice addition.

The 10EMOBJ emulsion had a lower pH value than 5EMOBJ whereas EMO has a higher value than EMOBJ (Table 3.4), similarly pH of salmon oil emulsions increased in order of 10ESOBJ, 5ESOBJ and ESO respectively (Table 3.5). However emulsion stability was not affected by the pH of the emulsions, indicating starch emulsifier (OSA starch) was not affected by the pH of the solution. But emulsions stabilized using protein emulsifiers are sensitive to the pH of the solution.

Table 3.5 Emulsion stability, pH and colour of SO emulsions

		ESO	5ESOBJ	10ESOBJ
% Emulsion stability		86.56±0.82[a]	89.93±0.91[a]	87.23±1.13[a]
pH		4.83±0.05[a]	4.31±0.03[b]	4.16±0.08[c]
color	L	85.37±0.38[a]	82.25±0.17[b]	80.94±0.12[c]
	a*	-0.37±0.17[c]	1.53±0.26[b]	2.91±0.07[a]
	b*	10.61±2.45[a]	4.97±0.11[b]	1.99±0.09[c]
hue angle		99.33±0.31[a]	75.61±0.62[b]	33.19±0.96[c]
Chroma		10.88±2.50[a]	5.17±0.10[b]	3.65±0.10[c]

Values are means and SD of triplicate determinations. [a-c]Means with different exponents in the each row indicate significant difference ($p \leq 0.05$).
SO=salmon oil; ESO= salmon oil emulsion; 5ESOBJ=salmon oil emulsion with 5% blueberry juice; 10ESOBJ=salmon oil emulsion with 10% blueberry juice.

Blueberry extract significantly change the emulsion color from off white to light pink color. 10EMOBJ and 5EMOBJ had higher a* and lower L* & b* values compared to EMO, and 10ESOBJ and 5ESOBJ had higher a* and lower L* and b* values compared to ESO. In most cases emulsion color is not a critical parameter, since the final product is intended to be used for food enrichment.

3.3.4 Rheological Properties of Emulsions

- Flow Behavior of Emulsions

Flow behaviors of feed emulsions are important for the spray drying operation. The emulsion viscosity is important to determine the particle size of the powders. More viscous emulsions produce larger droplet size while atomization produces powders with large particle size. The flow index (n) of emulsion EMO, 5EMOBJ and 10EMOBJ were less than 1 at all of the temperatures used, indicating a pseudo plastic fluid behavior (Table 3.6). Correspondingly n value of ESO, 5ESOBJ and 10ESOBJ were less than 1, representing a pseudo plastic fluid (Table 3.7).

Table 3.6 Flow behavior properties of EMO, 5EMOBJ and 10EMOBJ.

Temp.	n			k ($Pa.^n$)		
°C	EMO	5EMOBJ	10EMOBJ	EMO	5EMOBJ	10EMOBJ
5	0.770± 0.018^{cA}	0.695± 0.008^{cB}	0.770± 0.015^{cA}	0.137± 0.009^{aB}	0.264± 0.014^{aA}	0.137± 0.007^{aB}
15	0.827± 0.024^{bA}	0.717± 0.002^{bC}	0.797± 0.002^{bB}	0.047± 0.005^{bC}	0.183± 0.005^{bA}	0.094± 0.002^{bB}
25	0.912± 0.005^{aA}	0.763± 0.003^{aC}	0.819± 0.003^{aB}	0.024± 0.001^{cC}	0.118± 0.002^{cA}	0.065± 0.003^{cB}

Values are means and SD of triplicate determinations. $^{a-c}$Means with different exponents in the each column indicate significant difference ($p \leq 0.05$). $^{A-C}$Means with different exponents in the each row indicate significant difference ($p \leq 0.05$). EMO= menhaden oil emulsion; 5EMOBJ= menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice. n = flow index; k =consistency index.

The flow index of all emulsions increased with increased temperature. Thus emulsions attempt to behave as Newtonian fluids at higher temperatures. EMO shows higher flow index

compare to 5EMOBJ and 10EMOBJ. This may be due to the fact that SEMOBJ and 10EMOBJ has lower moisture content compared to EMO.

Table 3.7 Flow behavior properties of ESO, 5ESOBJ and 10ESOBJ.

Temp.	n			k (Pa.^{n})		
°C	ESO	5ESOBJ	10ESOBJ	ESO	5ESOBJ	10ESOBJ
5	0.788± 0.002^{bA}	0.683± 0.001^{bB}	0.392± 0.001^{bC}	0.117± 0.051^{aC}	0.246± 0.007^{aB}	2.941± 0.046^{aA}
15	0.793± 0.008^{bA}	0.700± 0.013^{bB}	0.400± 0.032^{bC}	0.100± 0.044^{bB}	0.211± 0.021^{bC}	2.212± 0.0452^{bA}
25	0.815± 0.004^{aA}	0.719± 0.002^{aB}	0.493± 0.031^{aC}	0.078± 0.034^{cB}	0.159± 0.006^{cC}	1.14± 0.065^{cA}

Values are means and SD of triplicate determinations. $^{a-c}$Means with different exponents in the each column indicate significant difference ($p \leq 0.05$). $^{A-C}$Means with different exponents in the each row indicate significant difference ($p \leq 0.05$). ESO= salmon oil emulsion; 5ESOBJ= salmon oil emulsion with 5% blueberry juice; 10ESOBJ = salmon oil emulsion with 10% blueberry juice; n = flow index; k =consistency index.

Conversely, the emulsion consistency index (k) decreased with increased temperature in all the emulsions. A higher k value in emulsions indicates a more viscous consistency (Bechtel and others 2009). Among different emulsions, 5EMOBJ showed higher k value than 10EMOBJ. EMO showed the lowest k value (Table 3.6). The k value of 10ESOBJ was greater than 5ESOBJ, and ESO has the lowest k value (Table 3.7). The higher k value may be due to the solids present in the juice. But 5EMOBJ having highest k value, does not show any clear relationship between total solids with the k value.

According to the Table 3.8 and 3.9, apparent viscosity of the emulsions decreased with increasing temperature. Emulsions viscosity is important during microencapsulation since it has a direct effect on emulsions droplet size during atomization (Hui and others 2010; Bechtel and others 2011).

Table 3.8 Apparent viscosity change of EMO, 5EMOBJ and 10EMOBJ with temperature

Temp. °C	Apparent viscosity (Pa.s)		
	EMO	5EMOBJ	10EMOBJ
5	0.023 ± 0.002^{aC}	0.040 ± 0.001^{aB}	0.053 ± 0.000^{aA}
15	0.019 ± 0.000^{bC}	0.032 ± 0.000^{bB}	0.041 ± 0.001^{bA}
25	0.011 ± 0.001^{cC}	0.014 ± 0.000^{cB}	0.016 ± 0.000^{cA}

Values are means and SD of triplicate determinations. $^{a-c}$Means with different exponents in the each column indicate significant difference ($p \leq 0.05$). $^{A-C}$Means with different exponents in the each row indicate significant difference ($p \leq 0.05$). EMO= menhaden oil emulsion; 5EMOBJ= menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice.

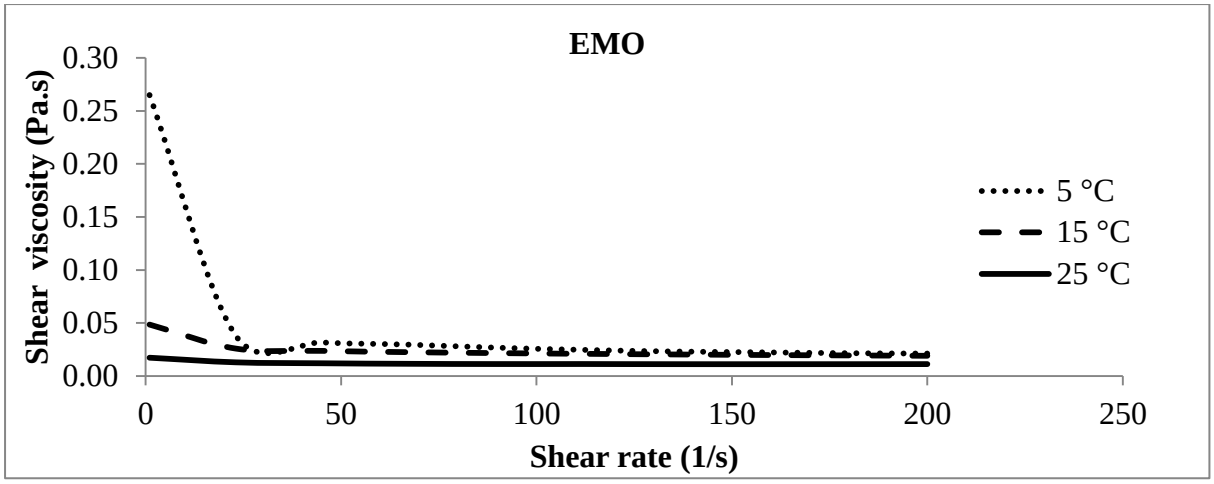
Table 3.9 Apparent viscosity change of ESO, 5ESOBJ and 10ESOBJ with temperature

Temp. °C	Apparent viscosity (Pa.s)		
	ESO	5ESOBJ	10ESOBJ
5	0.042 ± 0.002^{aC}	0.052 ± 0.002^{aB}	1.046 ± 0.042^{aA}
15	0.034 ± 0.002^{bC}	0.044 ± 0.001^{bB}	0.096 ± 0.005^{bA}
25	0.030 ± 0.000^{cC}	0.038 ± 0.001^{cB}	0.082 ± 0.001^{cA}

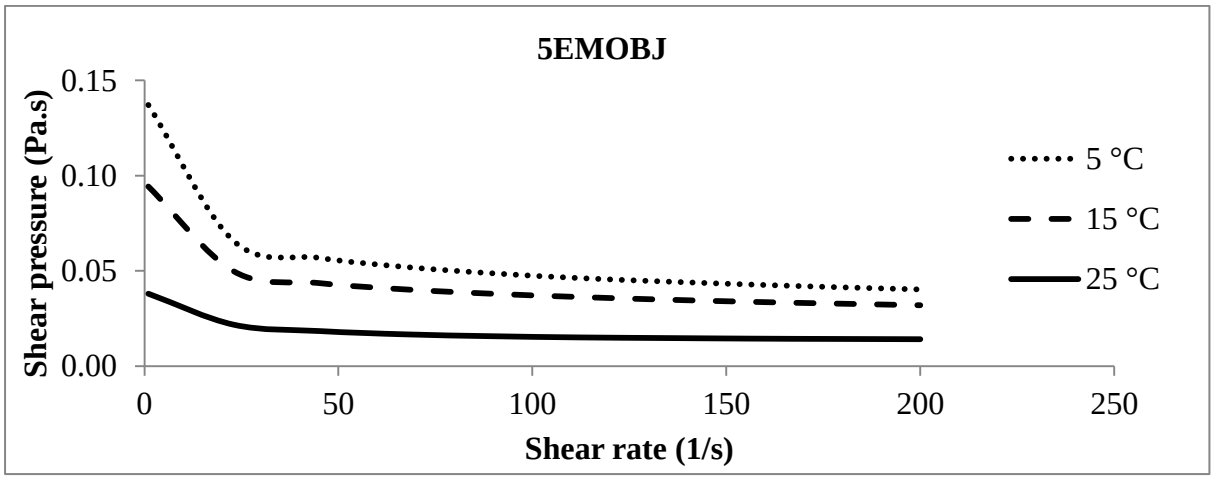
Values are means and SD of triplicate determinations. $^{a-c}$Means with different exponents in the each column indicate significant difference ($p \leq 0.05$). $^{A-C}$Means with different exponents in the each row indicate significant difference ($p \leq 0.05$). ESO= salmon oil emulsion; 5ESOBJ= salmon oil emulsion with 5% blueberry juice; 10ESOBJ = salmon oil emulsion with 10% blueberry juice.

The apparent viscosity at a shear rate of 200 s^{-1} was highest in 10EMOBJ emulsion compared to 5EMOBJ and EMO, designating more pseudo plastic behavior. In salmon oil emulsions, 10ESOBJ showed greater viscosity than 5ESOBJ.

According to Figure 3.2 and 3.3, all of the emulsions showed a decrease in viscosity with increased shear rate. Inversely they will increase in viscosity with decreased shear rate indicating alignment of the long polymer chains along the flow lines.



(a)



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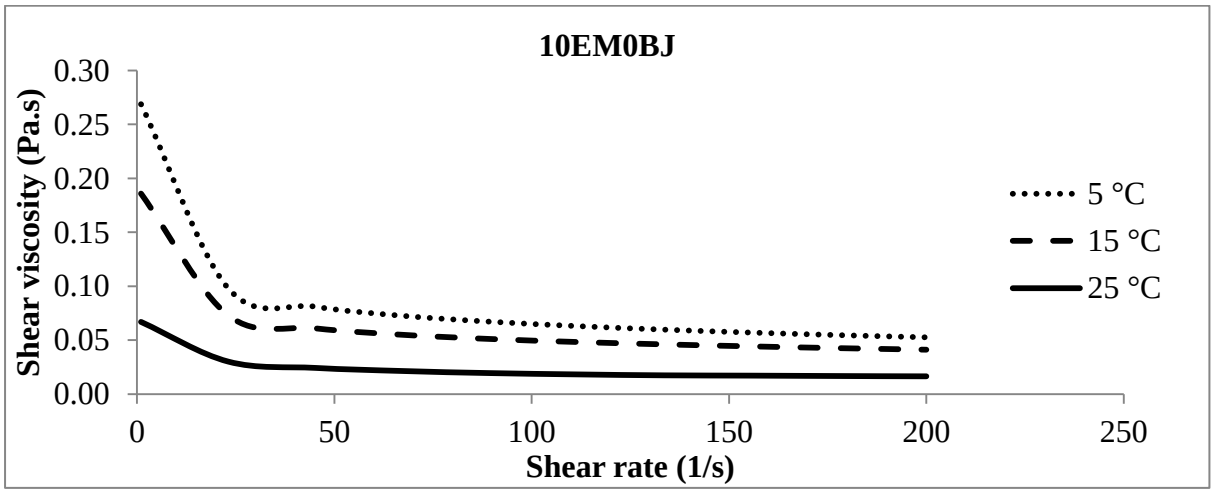


(c)

Figure 3.2 shear viscosity as a function of shear rate in emulsions of EMO, 5EMOBJ and 10EMOBJ (a, b and c respectively).

Values are means and SD of triplicate determinations. EMO = menhaden oil emulsion; 5EMOBJ = menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice.

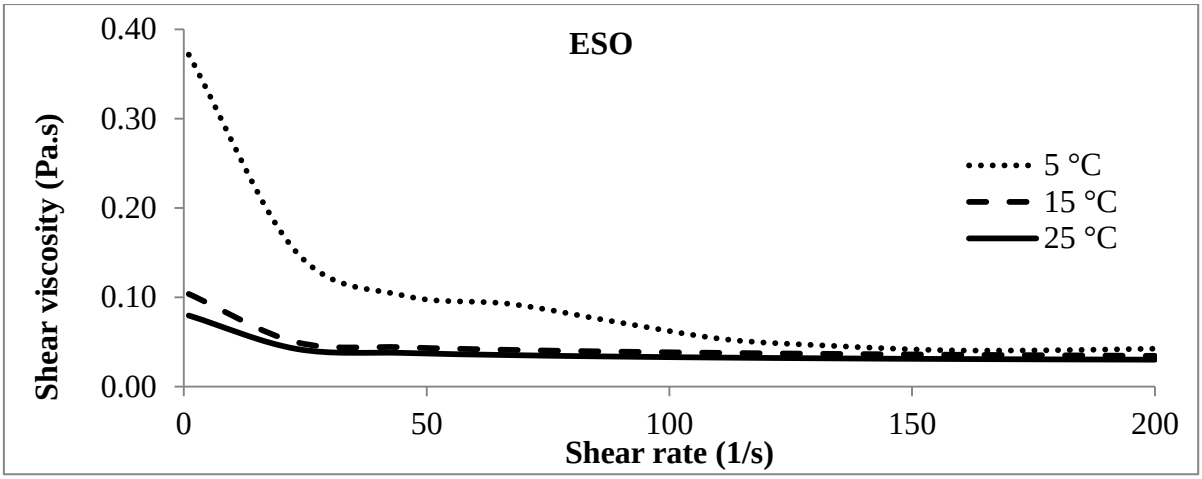
Figure 3.2 and 3.3, illustrates the shear thinning behavior of all three emulsions where EMO and ESO showed significant shear thinning at 5 ºC. But EMO and ESO shear thinning behavior at 15 and 25 ºC was not significant. Shear thinning fluids decrease in viscosity with increased shear rate. But with increased temperature shear thinning behavior delayed in all emulsions. The shear-thinning region is a consequence of a dramatic shear-induced structural breakdown, related to a mechanism of oil droplet deflocculation occurring in concentrated emulsions.

- Viscoelastic Properties of Emulsions

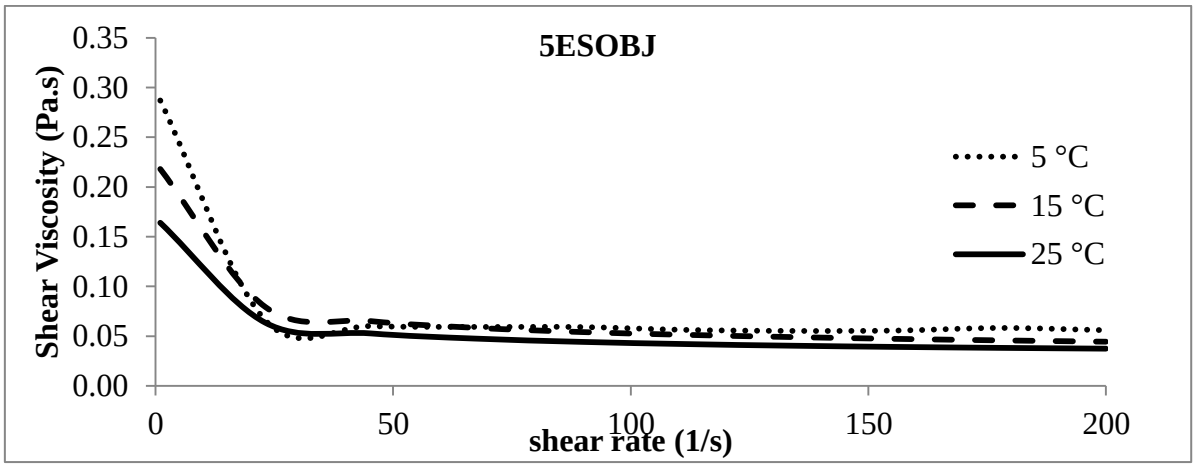
A fluid behaves as a liquid and a gel is referred to as a viscoelastic fluids. They can be pumped easily although they act as viscous liquids, and are able to suspend small solid particles owing to their elastic property. Viscoelastic property of an emulsion can be quantified using measurement of their G` (elastic or storage modulus) and G`` (viscous or loss modulus) as a function of frequency at a constant temperature. G' is a measure of energy recovered per cycle of sinusoidal shear deformation and G" is an estimate of energy dissipated as heat per cycle (Gunasekaran and Sun 2010; Bechtel and others 2011).

In other words G` measures the deformation energy of a sample during the shear process which represents elastic behavior, and G`` measures the deformation energy used up during the shear process and lost afterwards which represents viscous behavior. The material will behave like a solid when G` > G`` indicating the elastic nature with zero recovery. If G``> G`, material will behave like a liquid indicating viscous dissipation of used energy during deformation (Tabilo-Munizaga and Barbosa-Cànovas 2005).

Shift angle or delta degree is another property important in emulsions which measures energy loss (loss factor) compared to energy stored in each cycle of deformation (Bechtel and others 2011). The loss factor (delta angle) shows the ratio of viscous (G``) to the elastic (G`) of deformation behavior.



(a)



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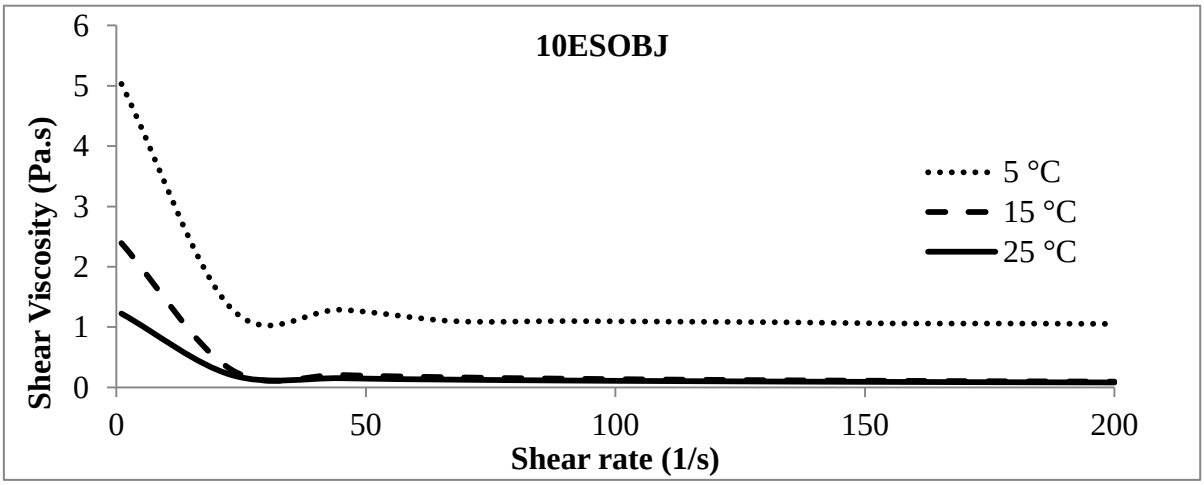


(c)

Figure 3.3 shear viscosity as a function of shear rate in emulsions of ESO, 5ESOBJ and 10ESOBJ (a, b and c respectively).

Values are means and SD of triplicate determinations. ESO= salmon oil emulsion; 5ESOBJ= salmon oil emulsion with 5% blueberry juice; 10ESOBJ = salmon oil emulsion with 10% blueberry juice.

The material is called viscoelastic when the loss factor is in between 0 and 90°. In pure viscous systems as water, the delta degree is 90º whereas purely elastic systems delta degree is 0º (Tadros 1994).

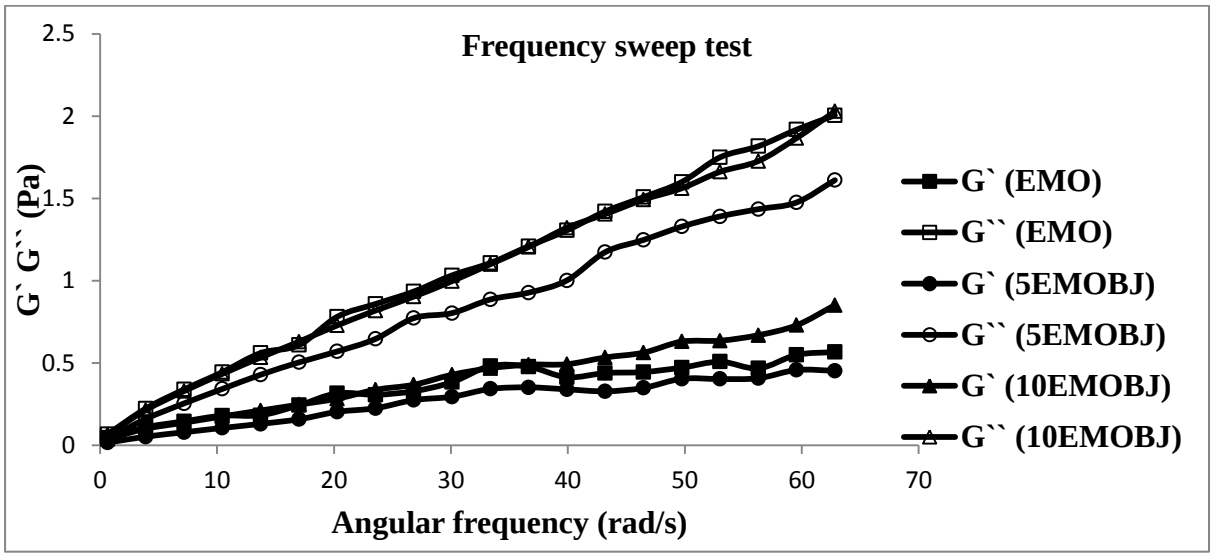


Figure 3.4 Viscoelastic properties of EMO, 5EMOBJ, and 10EMOBJ
Values are means and SD of triplicate determinations.
EMO = menhaden oil emulsion; 5EMOBJ = menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice; G' = storage modulus; G" = loss modulus.

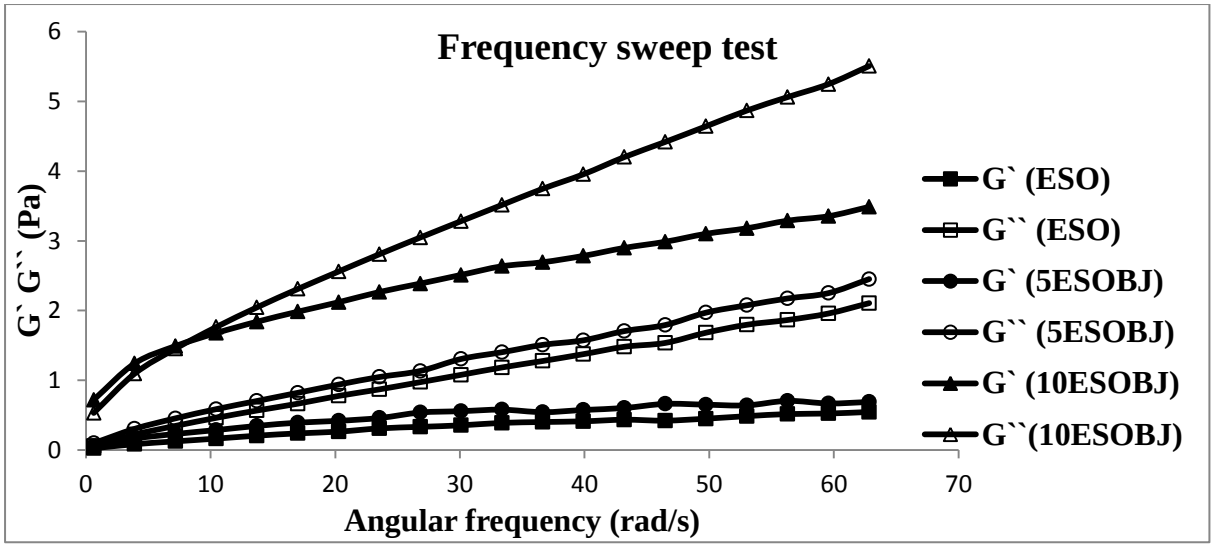


Figure 3.5 Viscoelastic properties of ESO, 5ESOBJ, and 10ESOBJ.
Values are means and SD of triplicate determinations.
ESO = salmon oil emulsion; 5ESOBJ = salmon oil emulsion with 5% blueberry juice; 10ESOBJ = salmon oil emulsion with 10% blueberry juice; G' = storage modulus; G" = loss modulus

Delta degree (loss factor) of EMO, 5EMOBJ and 10EMOBJ; ESO, 5ESOBJ and 10ESOBJ (Figure 3.6 and 3.7) lies between 0º and 90º indicating a viscoelastic behavior (Bechtel and others 2009). All three menhaden emulsions (EMO, 5EMOBJ and 10EMOBJ) show no significant difference in loss factor. All of the menhaden emulsions are viscoelastic. But the delta degree angle was around 60-70º in menhaden oil emulsions (Figure 3.6), which indicated more viscous behavior of emulsions compared to elastic behavior. However salmon oil emulsions showed increased elastic behavior with addition of BJ. 10ESOBJ had a lower loss factor compared to 5ESOBJ, and ESO had the highest loss factor (Figure 3.7).

All of the emulsions showed a higher G`` compared to G` due to presence of modified starch as the wall materials. Both G` and G`` rise in magnitude with increased angular frequency (Figure 3.4 and 3.5). Salmon oil emulsions showed increase G` and G`` with increased BJ addition, where menhaden emulsion showed no significant difference. Previous researchers reported a similar behavior in oil-in-water emulsions stabilized with starch. According to them, lowering the density difference between starch soluble water phase and oil phase, results in the loss modulus (G``) dominating the elastic modulus (G`) with increase in delta degree. The modified starch provides polymeric hindrance to the oil droplets without contributing to the elasticity of emulsions (Britten and others 2008). Also OSA emulsified starch is known to produce stable emulsions, and corn syrup is added as a bulking agent (Erdman and others 2005). The emulsions with visco-elastic properties are stable because they retard the rearrangement of macroscopic phase separation (Bechtel and others 2011).

3.3.5 Lipid Oxidation During Emulsification

The peroxide value (PV), p-anisidine value (AV) and Totox value of EMO, 5EMOBJ and 10EMOBJ after emulsification is shown in Table 3.10. When compared with initial MO condition, the emulsification process significantly increased the PV and AV in EMO and 5EMOBJ emulsions.

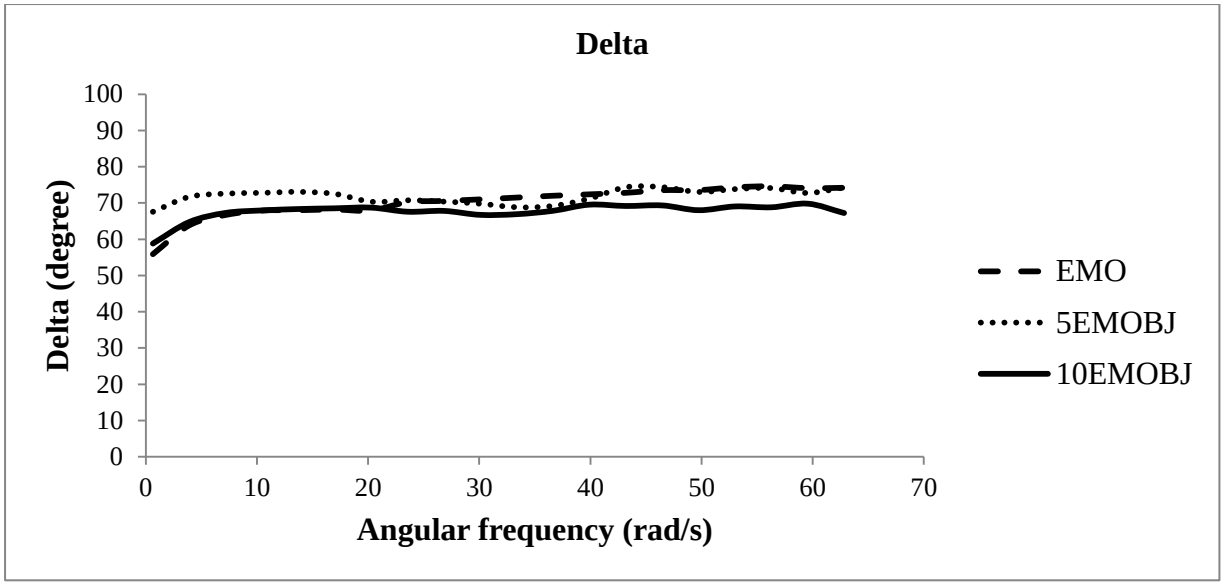


Figure 3.6 Rheology Properties of EMO, 5EMOBJ and 10EMOBJ: Phase Angle (δ) vs. Angular Frequency. Values are means and SD of triplicate determinations. EMO= menhaden oil emulsion; 5EMOBJ=menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice.

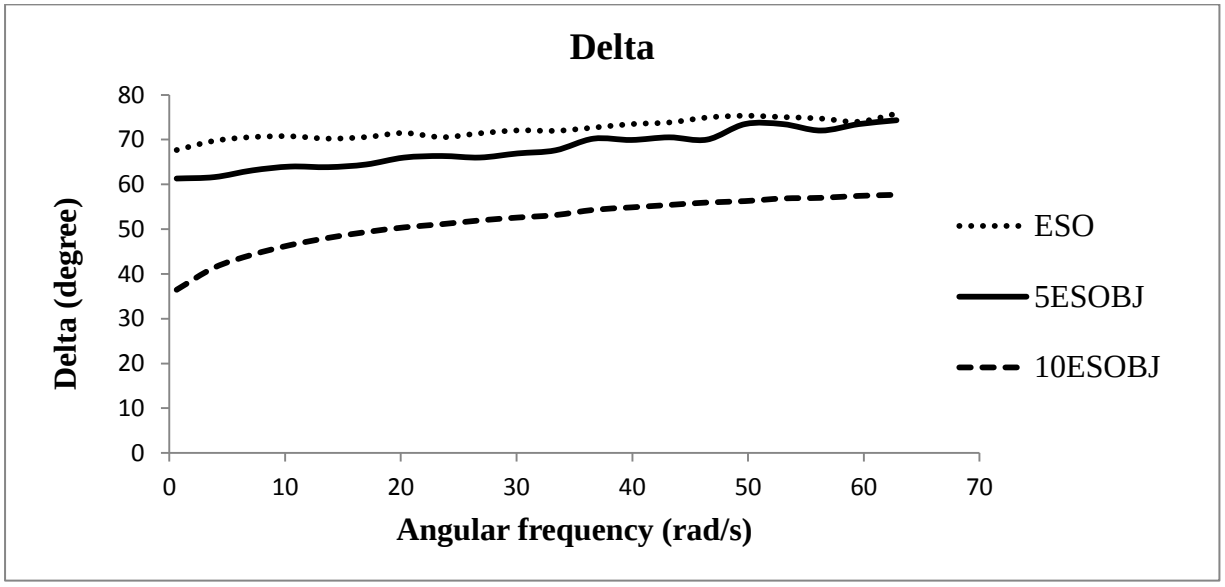


Figure 3.7 Rheology Properties of ESO, 5ESOBJ and 10ESOBJ: Phase Angle (δ) vs. Angular Frequency. Values are means and SD of triplicate determinations. ESO= salmon oil emulsion; 5ESOBJ=salmon oil emulsion with 5% blueberry juice; 10ESOBJ = salmon oil emulsion with 10% blueberry juice.

The PV value of EMO and 5EMOBJ increased from an initial value of 2.10 meq/kg to 2.78 and 2.56 respectively. But 10MOBJ did not show a significant difference in PV and AV compared to initial MO (Table 3.10). Likewise, the PV value of ESO and 5ESOBJ increased from the initial value 2.73 meq/Kg to 5.10 and 4.23 respectively, while 10ESOBJ did not show a significant difference (Table 3.11).

Also the AV value of these emulsions increased in a similar pattern. This indicates that blueberry juice at 10% addition effectively prevented the probable oxidation occurring during the emulsification process using the ultra-sonication technique. Also an adequate quantity of antioxidant compound is required to be present in order to have a significant effect on lipid oxidation.

Table 3.10 PV, AV, Totox value of MO and MO emulsions

	MO	EMO	5EMOBJ	10EMOBJ
PV[a]	2.10 ± 0.02^{c}	2.78 ± 0.02^{a}	2.56 ± 0.01^{b}	2.15 ± 0.03^{c}
AV	5.16 ± 0.06^{c}	5.87 ± 0.09^{a}	5.45 ± 0.04^{b}	5.22 ± 0.11^{c}
Totox	9.36 ± 0.38^{c}	11.43 ± 0.13^{a}	10.57 ± 0.06^{b}	9.52 ± 0.17^{c}

[a]meq peroxide /kg oil

Values are means and SD of triplicate determinations. [abc]Means with different exponents in the each row indicate significant difference ($p \leq 0.05$). MO= menhaden oil; EMO= menhaden oil emulsion; 5EMOBJ=menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice; PV=peroxide value; AV=anisidine value.

During microencapsulation fish oil undergoes multiple changes in physical properties such as bulk oil, dispersed oil in aqueous phase (emulsion), and dispersed oil in dry matrix (microencapsulated powders). Much previous research work has demonstrated occurrence of auto-oxidation during emulsification and spray drying.

Table 3.11 PV, AV, Totox value of SO and SO emulsions

	SO	ESO	5ESOBJ	10ESOBJ
PV[a]	2.73±0.59[d]	5.10±0.21[a]	4.23±0.18[b]	3.50±0.32[c]
AV	9.30±0.15[d]	12.43±0.17[a]	11.76±0.14[b]	10.03±0.11[c]
Totox	14.76±2.76[c]	22.63±0.59[a]	20.22±0.50[a]	17.03±0.75[b]

[a]meq peroxide /kg oil

Values are means and SD of triplicate determinations. [abcd]Means with different exonents5 in the each row indicate significant difference ($p \leq 0.05$). SO= menhaden oil; ESO= salmon oil emulsion; 5ESOBJ=salmon oil emulsion with 5% blueberry juice; 10ESOBJ = salmon oil emulsion with 10% blueberry juice; PV=peroxide value; AV=anisidine value.

Although microencapsulation is done to prevent lipid oxidation, addition of antioxidants is required to prevent lipid oxidation that occurs during processing and storage (Drusch and others 2007). Various studies attempted to study the effect of antioxidant addition on emulsification and subsequent spray drying during microencapsulation.

A study conducted using antioxidants such as tocopherol and ascorbyl palmitate showed a decrease in lipid oxidation during the emulsification process compared to control samples. But they did not show any significant effect during spray drying. However the emulsions with tocopherol and rosemary extract showed a reduction of auto-oxidation during the process of spray drying (Drusch and others 2009a; Hogan and others 2003). This could be explained by studies related to spray drying emulsions, where oil-in-water emulsions are better stabilized by lipophilic antioxidants such as tocopherol compare to hydrophilic antioxidants for example rosemary extract. Conversely, hydrophilic antioxidants may be more effective in bulk oil conditions as spray dried powders. Since blueberry extract consists of both lipophilic and hydrophilic antioxidants, it has the possibility of preventing lipid oxidation during the emulsification and spray drying processes. Moreover having larger quantities of hydrophilic antioxidant activity over lipophilic, blueberry extract can effectively

prevent lipid oxidation in the microencapsulated powders during spray drying and subsequent storage.

Also different studies observed high levels of tocopherol and lycophene addition were related to an increase in lipid oxidation (Kolanowski and others 2006). High levels of tocopherol promote hydroperoxide formation through accumulation of tocopherol radicals which have been shown to be unsuitable to stabilize fish oil emulsions rich in polyunsaturated fatty acids (Drusch and others 2009a; Beindroff and Zidam 2010). But antioxidants such as polyphenols do not produce free radicals since the molecule can stabilize itself after neutralization of free radicals.

The emulsification method used in the experiment i.e. ultra-sonication is known to cause lipid oxidation. Chemat and others (2004) reported an increase in PV value from 5.38 meq/kg to 6.33 meq/kg in sonicated sunflower oil samples processed for 2 min (20 KHz, 150 W). The ultrasound assisted lipid oxidation is attributed to the cavitation phenomenon, which affected structural and functional components leading to lipid oxidation. The collapse of cavitation bubbles in the emulsions result in formation of energy accumulated hot spots having temperatures above 5000 ºC and pressure of 500 MPa which caused lipid oxidation by mechanisms like the thermal effect, due to free radicals generated during sonication and mechanical forces created by micro-streaming and shock waves.

Furthermore intense mechanical stress and agitation owing to shear and turbulence during emulsification lead to oxygen inclusion and distribution within the emulsions which will increase the rate of lipid oxidation (Drusch and others 2009a; Dunford and Legako 2010). Furthermore it has shown that oxygen availability in the emulsion related to an increase in lipid oxidation during the time of spray drying rather than in drying gas (Drusch and Mannino 2009b).

3.3.6 ME of Microencapsulated MO and SO Powders

Table 3.12 shows the surface oil content, total oil content and microencapsulation efficiency (ME) of microencapsulated powders of menhaden oil and salmon oil. The high oil load was used in the experiment in order to study the effect of extract antioxidant on lipid oxidation at worst possible conditions. The total oil content of menhaden oil and salmon oil powders were 55-59% and 52-53% respectively.

Table 3.12. Surface oil content, total oil content and microencapsulation efficiency of MO and SO microencapsulated powders

Powder properties	MO			SO		
	MMO	5MMOBJ	10MMOBJ	MSO	5MSOBJ	10MSOBJ
Surface oil content (g/g)	0.072± 0.001^{a}	0.036± 0.003^{c}	0.059± 0.001^{b}	0.098± 0.001^{x}	0.085± 0.002^{z}	0.089± 0.003^{y}
Total oil content (g/g)	0.59± 0.02^{a}	0.57± 0.04^{a}	0.55± 0.01^{a}	0.52± 0.03^{x}	0.53± 0.02^{x}	0.53± 0.03^{x}
Microencapsulation efficiency %	87.80± 0.92^{b}	93.68± 0.66^{a}	89.27± 0.60^{b}	81.15± 1.36^{y}	83.96 ± 0.98^{x}	83.20± 0.12^{x}

Values are means and SD of triplicate determinations. $^{a-c}$Means and ^{xy}means with different exponents in the each row indicate significant difference ($p \leq 0.05$).
MO= menhaden oil; SO = salmon oil; EMO= menhaden oil emulsion; 5EMOBJ=menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice; ESO= salmon oil emulsion; 5ESOBJ=salmon oil emulsion with 5% blueberry juice; 10ESOBJ = salmon oil emulsion with 10% blueberry juice.

There was no significant difference of total oil content in menhaden oil MMO, 5MMOBJ and 10MMOBJ powders. Similarly salmon oil MSO, 5MSOBJ and 10MSOBJ shows no significant difference in total oil content. But surface oil content of MMO was

significantly higher than 10MMOBJ and 5MMOBJ. 5MMOBJ showed the least surface oil content compared to other menhaden oil powders.

Likewise, salmon oil powders showed higher surface oil content in MSO sample where 5MSOBJ showed the least amount of surface oil. All of the menhaden oil powders showed greater microencapsulation efficiency. 5MMOBJ showed significantly higher encapsulation efficiency than MMO and 10MMOBJ. MMO and 10MMOBJ showed no significant difference in microencapsulation efficiency. On the other hand salmon oil powders showed less encapsulation efficiency compared to menhaden powders with encapsulation efficiency between 81-83%. The 10MSOBJ and 5MSOBJ had significantly higher encapsulation efficiency than MSO. The lower ME in salmon oil powders is attributed to the purity of the initial oil. Salmon crude oil was purified using 5% activated earth to separate the oil from the other impurities. But this procedure did not follow proper oil purification which will cause retention of some impurities in the purified oil. These impurities might affect the microencapsulation process leading to lowered ME.

Successful microencapsulation of fish oil should result in higher microencapsulation efficiency with low minimum surface oil content on the powder particles. The surface oil contents not only effect the wettability and dispensability of the powder but also readily undergoes lipid oxidation (Assadpoor and others 2008a). Microencapsulation efficiency can be affected by many factors such as properties of wall and core materials, emulsion characteristics and spray drying parameters (Bhandari and others 2007a; Assadpoor and others 2008b). Generally microencapsulated powder has oil load percentages ranging from 30-50%. Having higher oil load in the microcapsules is advantageous. But high oil load in microcapsules is associated with reduced encapsulation efficiencies and increased surface oil contents (Chan and others 2009). It has been suggested that core to wall material ratio of 1:4 is optimal for wall materials like gum arabic and modified starches (Bhandari and others

2007b). However the microcapsules produced using n-OSA emulsifying starch and corn syrup with an oil load of 57% in menhaden oil powders, and 52% in salmon oil powders, produced powders with higher ME. Changing the core material and wall material of these powder formulations could produce powders with 100% ME.

Some previous research work has shown that feed solid content has an impact on ME. Higher emulsion solid content will increase core material retention by reducing the time taken to form the semi permeable membrane at the surface of drying powder particles. This will increase the rapid skin formation around the microcapsules (Bhandari and others 2007b). But higher feed solid content will increase the emulsion viscosities, and create larger droplets corresponding to increased particle size of microcapsules. During the experiment n-OSA starch was used in combination with corn syrup as wall materials because high dextrose equivalent (DE) corn syrup is less permeable to oxygen and offers better protection to encapsulated oil.

3.3.7 Color of Microencapsulated Powders

Table 3.13 Color of microencapsulated MO and SO powders

	MO			SO		
	MMO	5MMOBJ	10MMOBJ	MSO	5MSOBJ	10MSOBJ
L	97.03 ± 0.03^{a}	89.33 ± 0.02^{b}	83.41 ± 0.02^{c}	91.86 ± 1.97^{x}	88.04 ± 2.15^{y}	80.23 ± 3.52^{z}
a*	-1.04 ± 0.01^{c}	3.42 ± 0.01^{b}	6.13 ± 0.01^{a}	0.54 ± 0.09^{z}	2.18 ± 0.22^{y}	5.68 ± 0.93^{x}
b*	5.24 ± 0.03^{a}	1.27 ± 0.03^{b}	1.07 ± 0.01^{c}	9.48 ± 1.03^{x}	5.45 ± 0.92^{y}	3.45 ± 0.84^{z}

Values are means and SD of triplicate determinations. ^{abc}Means and ^{xy}means with different exonents in the each row indicate significant difference ($p \leq 0.05$).
MO= menhaden oil; SO = salmon oil; MMO= microencapsulated menhaden oil; 5MMOBJ=microencapsulated menhaden oil with 5% blueberry juice; 10MMOBJ = microencapsulated menhaden oil with 10% blueberry juice; MSO= microencapsulated salmon oil; 5MSOBJ=microencapsulated salmon oil with 5% blueberry juice; 10MSOBJ = microencapsulated salmon oil with 10% blueberry juice.

The L*, a* and b* color values of microencapsulated menhaden oil powders (MMO, 5MMOBJ and 10MMOBJ) and salmon oil powders (MSO, 5MSOBJ and 10MSOBJ) are shown in Table 3.13.

The color of the emulsions with or without the blueberry juiceb correlated with the color of microencapsulated powders. Blueberry juice addition increase a* value of the powders and reduce the L* and b* values compared to the powder without adding the juice. The color change of the menhaden and salmon oil powders is better illustrated by Figure 3.8 and 3.9 respectively. The powders produced without the juice showed light yellow color (higher b*) due to the color of the oil.

(a) (b) (c)

Figure 3.8 Image of microencapsulated 10MMOBJ, 5MMOBJ and MMO powders (a, b, and c respectively).
EMO= menhaden oil emulsion; 5EMOBJ=menhaden oil emulsion with 5% blueberry juice; 10EMOBJ = menhaden oil emulsion with 10% blueberry juice.

(a) (b) (c)

Figure 3.9 Image of microencapsulated 10MSOBJ, 5MSOBJ and MSO powders (a, b, and c respectively).
ESO= microencapsulated salmon oil; 5ESOBJ=microencapsulated salmon oil with 5% blueberry juice; 10ESOBJ = microencapsulated salmon oil with 10% blueberry juice.

3.3.8 Lipid Oxidation During Microencapsulation

After emulsion is sprayed into the drying chamber, the temperature of the droplet surface will be at the wet bulb temperature correspond to the drying temperature during the initial stages of drying. In most cases droplet temperature does not exceed 100 ºC (Chambin and others 2007). Mostly this temperature is indicated by the air exhaust temperature, which is typically around 50-80 ºC. During the drying process a film is formed at the surface of the drying particle (Beindroff and Zidam 2010).

Many authors argue that lipid oxidation is slowed down in a low moisture environment. But conversely some researchers reported that extreme dry conditions accelerate lipid oxidation (Baik and others 2004). But more rapid oxidation was observed in the intermediate moisture content level.

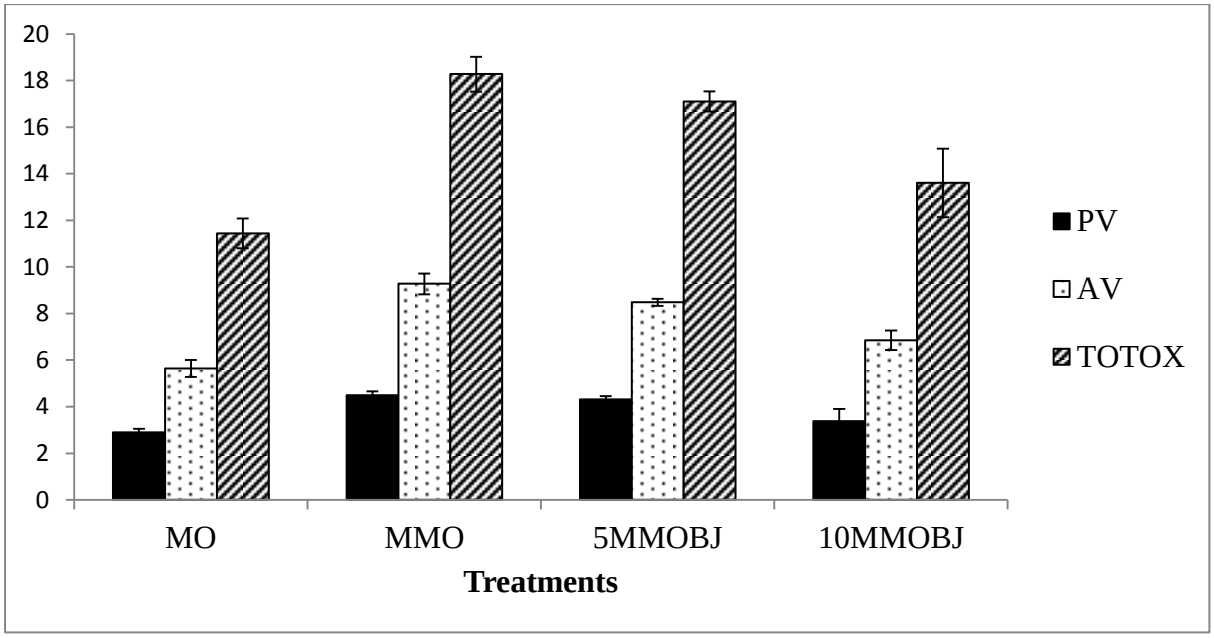


Figure 3.10 PV, AV, Totox value of MMO, 5MMOBJ and 10MMOBJ powders.
Values are means and SD of triplicate determinations. MO= menhaden oil; MMO= microencapsulated menhaden oil; 5MMOBJ=microencapsulated menhaden oil with 5% blueberry juice; 10MMOBJ = microencapsulated menhaden oil with 10% blueberry juice; PV=peroxide value; AV=anisidine value.

Baik and others (2004) reported increase of PV of fish oil emulsions from 9.5±0.4 mmol/Kg to 17.4±4.0 mmol/Kg in the spray dried powders (Drusch and others 2009a; Drusch and others 2006a). They explained that during the spray drying process, fish oil exposed to air, high temperature and high pressure leads to increase lipid oxidation (Franke and Heinzelmann 1999). Similarly, our experimental results showed an increase in PV and AV in microencapsulated menhaden and salmon oil powders compared to pure oil without the addition of blueberry juice (Figure 3.10 and 3.11).

According to Figure 3.10, 10MMOBJ significantly had lower PV, AV and Totox value compared to 5MMOBJ and MMO powders. This indicated that 10MMOBJ with 10% juice addition prevented the lipid oxidation during spray drying. Correspondingly, 5MMOBJ significantly lowered PV, AV and Totox value compared to MMO. This showed that increased amount of juice addition reduced lipid oxidation during spray drying. But 10MMOBJ had significantly higher lipid oxidation values compared to initial menhaden oil.

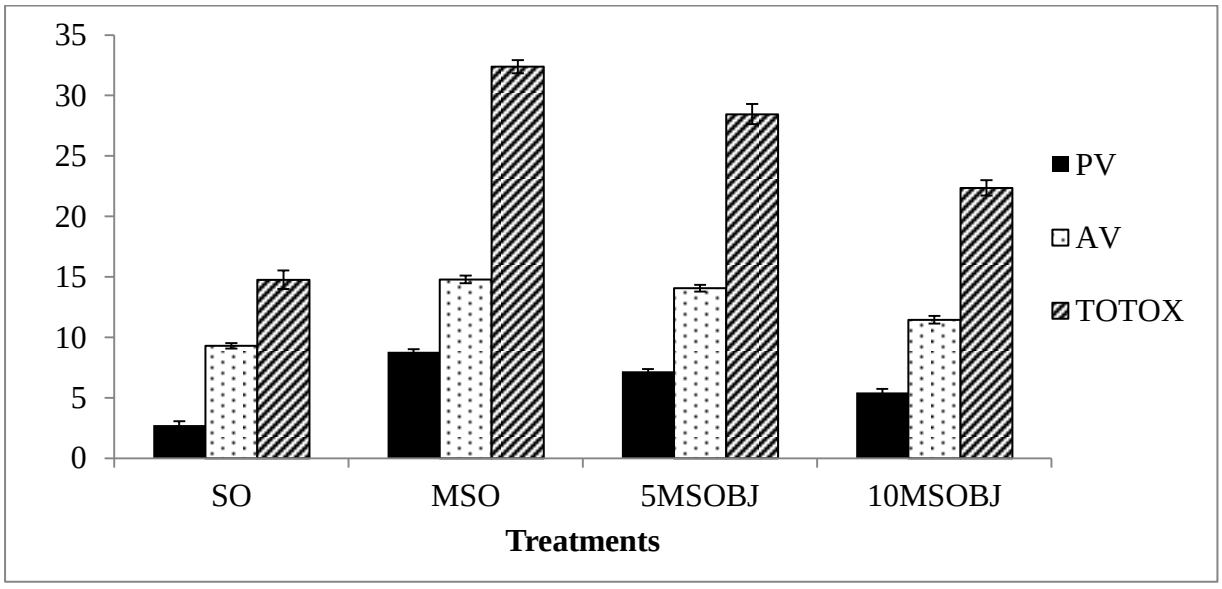


Figure 3.11 PV, AV, Totox value of MSO, 5MSOBJ and 10MSOBJ powders.
Values are means and SD of triplicate determinations. SO=salmon oil; MSO= microencapsulated salmon oil; 5MSOBJ=microencapsulated salmon oil with 5% blueberry juice; 10MSOBJ = microencapsulated salmon oil with 10% blueberry juice; PV=peroxide value; AV=anisidine value.

Similarly, 10MSOBJ showed significantly reduced PV, AV and Totox values compare to other salmon oil powders, while 10MSOBJ significantly showed lower values compared to MSO. According to these results, blueberry juice antioxidants effectively reduced lipid oxidation during spray drying. But further studies are necessary to establish most appropriate concentrations of the juice need to prevent lipid oxidation without having significant difference from the initial oil content.

The lipid oxidation during spray drying depends on drying temperature where increased drying temperature related to increased lipid oxidation (Kolanowski and others 2006). Furthermore the fraction of un-encapsulated oil i.e. surface oil might undergo rapid oxidation during the spray drying process (Drusch and others 2006a).

3.3.9 FAME Composition of MO, SO and Microencapsulated Powders

The Table 3.14 and 3.15 summarizes the percent of fatty acid methyl esters present in the initial MO and SO, and oil extracted out from microencapsulated powders of MMO, 5MMOBJ, 10MMOBJ and MSO, 5MSOBJ and 10MSOBJ.

The EPA and DHA omega-3 type fatty acids are the most important and unstable fatty acids present in menhaden and salmon oils, which can undergo quick oxidation due to their highly unsaturated nature. The experimental values reported for menhaden oil EPA and DHA content is in accordance with the literature. MO, MMO, 5MMOBJ and 10MMOBJ had a percent EPA content of 14.21, 13.94, 14.04 and 14.13 respectively (Table 3.14). The EPA content of MO and 10MMOBJ did not show significant difference while EPA content of MMO and 5MMOBJ showed a significant difference compared to MO.

But 5MMOBJ and MMO shows no significant difference in EPA content. The percent DHA contents of MO, MMO, 5MMOBJ and 10MMOBJ were 8.64, 7.75, 7.98 and 8.51 respectively. DHA content also showed a similar pattern to EPA, where MO showed no

difference from 10MMOBJ, while MO was significantly different from 5MMOBJ and MMO, and 5MMOBJ showed no difference from MMO.

Table 3.14 FAMEs Composition (% w/w) of MO, MMO, 5MMOBJ and 10MMOBJ.

	MO	10MMOBJ	5MMOBJ	MMO
C14:0 Myristic	8.73±0.07^{a}	8.88±0.07^{a}	8.81±0.06^{a}	8.86±0.06^{a}
C16:0 Palmitic	19.31±0.10^{b}	19.68±0.28^{a}	19.25±0.23^{b}	19.54±0.06^{a}
C16:1n7 palmitooleic	11.87±0.07^{a}	11.90±0.05^{a}	12.07±0.04^{a}	11.97±0.07^{a}
C18:0 Stearic	3.63±0.01^{a}	3.63±0.04^{a}	3.63±0.03^{a}	3.67±0.01^{a}
C18:1n9c Oleic	6.29±0.03^{a}	6.36±0.02^{a}	6.40±0.04^{a}	6.35±0.02^{a}
C18:1n5	3.11±0.02^{b}	3.14±0.01^{b}	3.17±0.01^{a}	3.14±0.01^{b}
C18:2n6c Linoleic	1.52±0.01^{a}	1.53±0.01^{a}	1.54±0.01^{a}	1.53±0.01^{a}
C18:3n3 Alpha-Linolenic	1.69±0.02^{a}	1.67±0.02^{a}	1.69±0.01^{a}	1.67±0.01^{a}
C20:1n9	0.99±0.00^{a}	1.01±0.02^{a}	1.03±0.03^{a}	1.02±0.01^{a}
C20:3n3	1.80±0.00^{b}	1.80±0.01^{a}	1.83±0.02^{a}	1.80±0.02^{a}
C20:4n6 arachidonic	1.15±0.06^{a}	1.16±0.02^{a}	1.17±0.01^{a}	1.15±0.01^{a}
EPA (20:5n3)	14.21±0.03^{a}	14.13±0.05^{a}	14.04±0.03^{b}	13.94±0.08^{b}
DHA (22:6n3)	8.64±0.20^{a}	8.51±0.08^{a}	7.98±0.08^{b}	7.75±0.03^{b}
C24:1n9	13.64±0.20^{a}	13.51±0.08^{a}	13.75±0.08^{a}	13.51±0.04^{a}
Total omega 3	24.54±0.05^{a}	24.31±0.03^{b}	23.71±0.08^{c}	23.36±0.06^{d}
Total omega 6	2.67±0.02^{b}	2.69±0.01^{b}	2.71±0.03^{a}	2.68±0.02^{a}
Saturates	33.24±0.51^{a}	33.80±0.80^{a}	33.28±0.77^{a}	33.67±0.81^{a}
Mono unsaturates (M)	36.28±0.19^{a}	36.30±0.13^{a}	36.80±0.18^{a}	36.37±0.13^{a}
Poly unsaturates (P)	29.01±0.27^{a}	28.80±0.19^{a}	28.25±0.15^{b}	27.84±0.16^{c}
ω3/ω6	9.19±0.03^{a}	9.04±0.01^{b}	8.75±0.02^{c}	8.72±0.01^{c}
P/S	0.87±0.02^{a}	0.85±0.02^{a}	0.85±0.05^{a}	0.83±0.03^{a}
Total fatty acids identified	98.53±0.12	98.90±0.08	98.38±0.11	97.88±0.12

Values are means and SD of triplicate determinations. $^{a\text{-}bc}$Means with different exponents in the each row indicate significant difference ($p \leq 0.05$). MO=menhaden oil; MMO= microencapsulated menhaden oil; 5MMOBJ=microencapsulated menhaden oil with 5% blueberry juice; 10MMOBJ = microencapsulated menhaden oil with 10% blueberry juice; FAMEs = fatty acid methyl esters, P/S = polyunsaturated fatty acids/ saturated fatty acids.

Table 3.15. FAMEs Composition (% w/w) of SO, MSO, 5MSOBJ and 10MSOBJ.

	SO	10MSOBJ	5MSOBJ	MSO
C14:0 Myristic	4.64±0.07[a]	4.35±0.04[a]	4.87±0.04[a]	4.82±0.11[a]
C16:0 Palmitic	14.53±0.25[b]	15.52±0.20[a]	15.21±0.24[a]	15.05±0.43[a]
C16:1n7 palmitooleic	5.60±0.05[a]	5.96±0.03[a]	5.92±0.04[a]	5.84±0.05[a]
C18:0 Stearic	3.00±0.05[a]	3.22±0.05[a]	3.15±0.05[a]	3.11±0.08[a]
C18:1n9c Oleic	18.19±0.15[a]	18.16±0.07[a]	18.06±0.13[b]	17.74±0.20[c]
C18:1n5	2.95±0.03[a]	2.84±0.02[b]	2.81±0.03[b]	2.77±0.03[c]
C18:2n6c Linoleic	1.38±0.01[a]	1.41±0.03[a]	1.42±0.01[a]	1.43±0.01[a]
C18:3n3 Alpha-Linolenic	1.20±0.04[a]	1.11±0.05[a]	1.14±0.02[a]	1.16±0.02[a]
C20:1n9	8.57±0.06[a]	8.54±0.01[a]	8.53±0.07[a]	8.44±0.11[b]
EPA (20:5n3)	10.33±0.05[a]	10.21±0.07[ab]	10.12±0.07[bc]	10.05±0.06[c]
DHA (22:6n3)	12.91±0.03[a]	12.89±0.03[a]	12.84±0.11[ab]	12.66±0.16[b]
C24:1n9	12.30±0.12[a]	12.39±0.03[a]	12.46±0.11[a]	12.42±0.16[a]
Total omega 3	24.44±0.11[a]	24.11±0.14[ab]	23.97±0.15[b]	23.73±0.13[c]
Total omega 6	1.45±0.02[a]	1.41±0.03[b]	1.42±0.02[b]	1.43±0.03[b]
Saturates	22.73±0.20[a]	23.68±0.17[a]	23.81±0.13[a]	23.55±0.24[a]
Mono unsaturates	47.80±0.11[a]	47.89±0.16[a]	47.78±0.12[b]	47.21±0.10[c]
Poly unsaturates	26.26±0.08[a]	25.52±0.08[b]	25.39±0.07[b]	25.16±0.10[c]
ω3/ω6	16.86±0.02[a]	17.10±0.01[a]	16.88±0.02[a]	16.59±0.01[b]
P/S	1.16±0.02[a]	1.08±0.01[a]	1.07±0.03[a]	1.07±0.02[a]
Total fatty acids identified	96.99±0.22	97.09±0.70	96.98±0.12	95.92±0.24

Values are means and SD of triplicate determinations. [a-c]Means with different exponents in the each row indicate significant difference ($p \leq 0.05$).

SO=purified salmon oil; MSO= microencapsulated salmon oil; 5MSOBJ=microencapsulated salmon oil with 5% blueberry juice; 10MSOBJ = microencapsulated salmon oil with 10% blueberry juice; FAMEs = fatty acid methyl esters, P/S = polyunsaturated fatty acids/ saturated fatty acids.

The values reported for total omega-3 fatty acid in menhaden oil showed agreement with the previous research work (Law and others 2001). Total omega-3 content of menhaden oils significantly decreased according to the sequence of MO, 10MMOBJ, 5MMOBJ and MMO (Table 3.14). But values reported for w3/w6 ratio were higher than the values reported from other studies. W3/w6 ratio did show significant difference between samples, while 10MMOBJ showed significant difference from 5MMOBJ, and 5MMOBJ did not show significant difference from MMO. Polyunsaturated to saturated ratio of all menhaden oil samples did not show any significant difference.

Similarly EPA content of SO and salmon oil powders MSO, 5MSOBJ and 10MSOBJ were 10.33, 10.05, 10.12 and 10.21 respectively (Table 3.15). MSO showed no significant difference from 10MSOBJ, and showed difference from 5MSOBJ and MSO. Also 10MSOBJ and 5MSOBJ did not show any difference, while 10MSOBJ showed significant difference from MSO. DHA content of MO, 10MSOBJ and 5MSOBJ did not show significant difference while MSO showed significant difference from other samples.

3.3.10 Particle Size of Powders

The particle size distribution of MMO, 5MMOBJ and 10MMOBJ microencapsulated powders are illustrated in the Figure 3.12. All of the powders had mean particle size ranging from 20 to 30 µm.

The particle size of the microcapsules generally varies between 10-400 µm. Addition of blueberry juice increased the mean particle size with increased juice concentration (Figure 3.12). Mean particle size of 10MMOBJ was significantly higher than MMO and 5MMOBJ, where 5MMOBJ showed greater mean particle size compared to MMO. But as shown in Figure 3.13, the mean particle size of MSO and 5MSOBJ did not show significant difference having a value ranging from 12-13 µm.

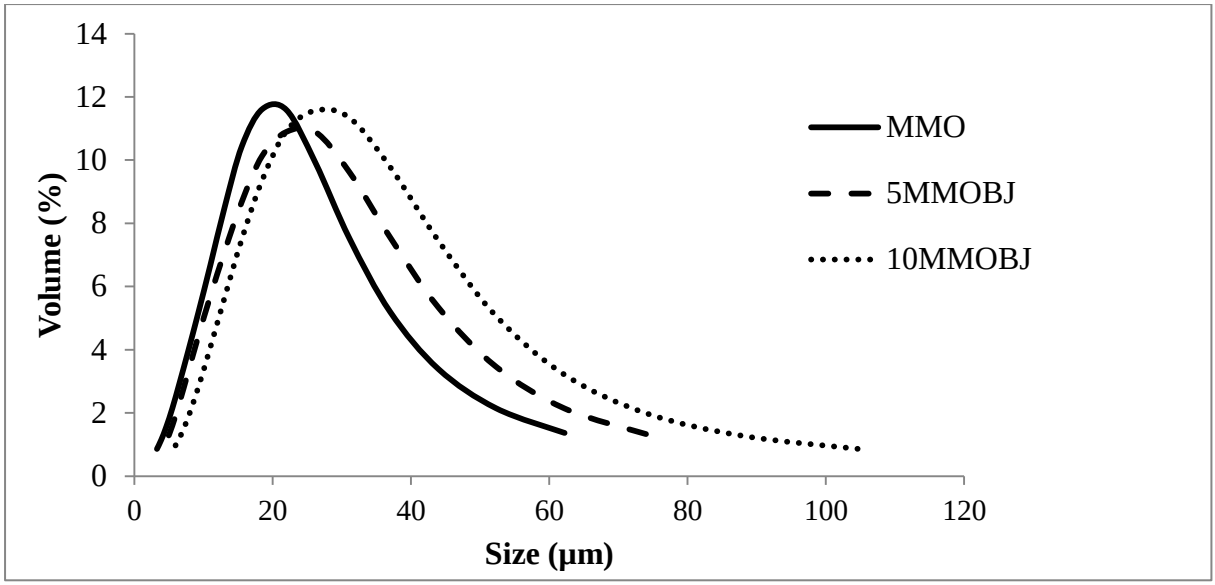


Figure 3.12. Particle size distribution of MMO, 5MMOBJ and 10MMOBJ.
Values are means and SD of triplicate determinations.
MMO= microencapsulated menhaden oil; 5MMOBJ=microencapsulated menhaden oil with 5% blueberry juice; 10MMOBJ = microencapsulated menhaden oil with 10% blueberry juice.

The mean particle size of 10MSOBJ showed slight increase compared to MSO and 5MSOBJ with a mean value of 15 µm. However all of the produced powders had mean particle size within a range of 15 to 30 µm. Some researcher's argue that higher particle size increased the amount of oil retention. Conversely, Chang et al. (1988) found that total oil retention was highest in intermediate size particles while large particles showed the lowest oil retention. Large particle sizes of microcapsules are known to be more storage stable than small particles (Beindroff and Zidam 2010). Also it was found that increased oil load in emulsions will form larger oil droplets corresponding to an increase in mean particle size of microcapsules. Likewise, larger particle size will retain more encapsulated oil (Berg and Drusch 2008).

Particle size can be affected by emulsion characteristics (viscosity and solid concentration) and drying conditions. Increase in drying air inlet and decrease in difference in inlet and outlet temperature have been shown to increase the particle size of the

microcapsules. This is mainly due to the fact that, higher drying rates set the particle sizes earlier and this will prevent the shrinkage of the particle drying.

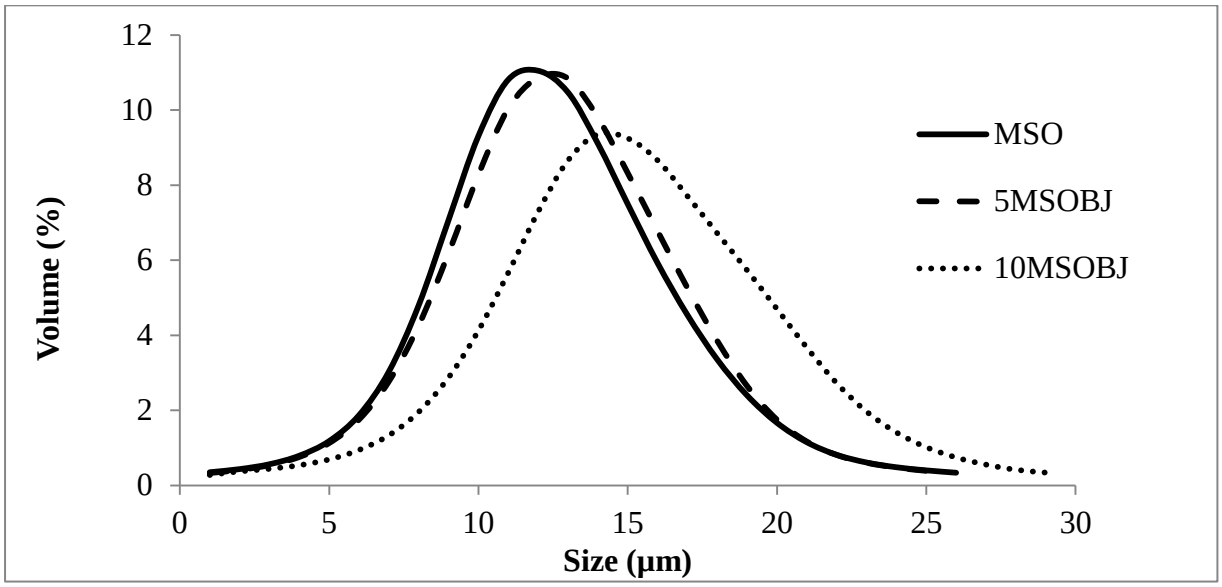
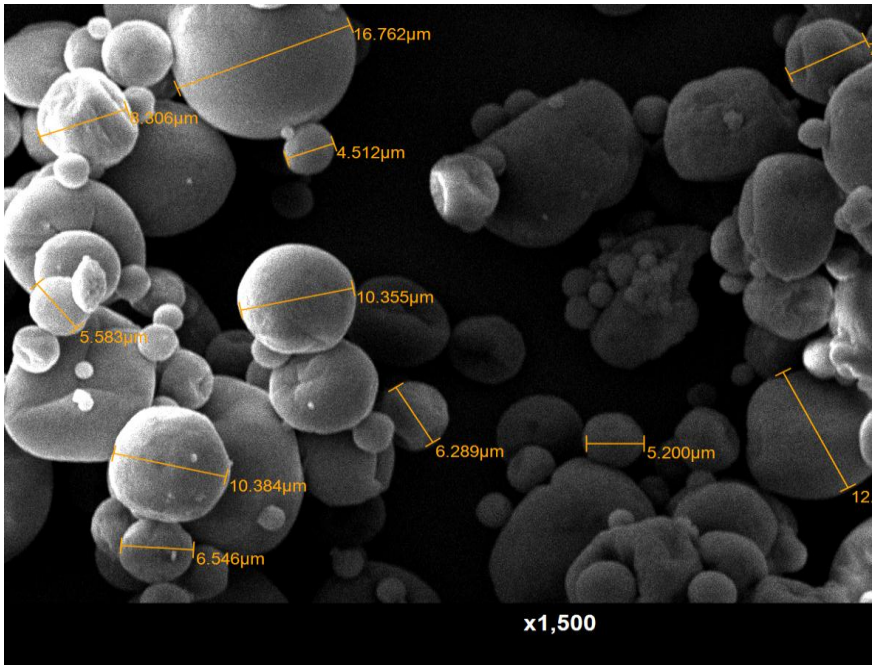


Figure 3.13 Particle size distribution of MSO, 5MSOBJ and 10MSOBJ.
Values are means and SD of triplicate determinations.
MSO= microencapsulated salmon oil; 5MSOBJ=microencapsulated salmon oil with 5% blueberry juice; 10MSOBJ = microencapsulated salmon oil with 10% blueberry juice.

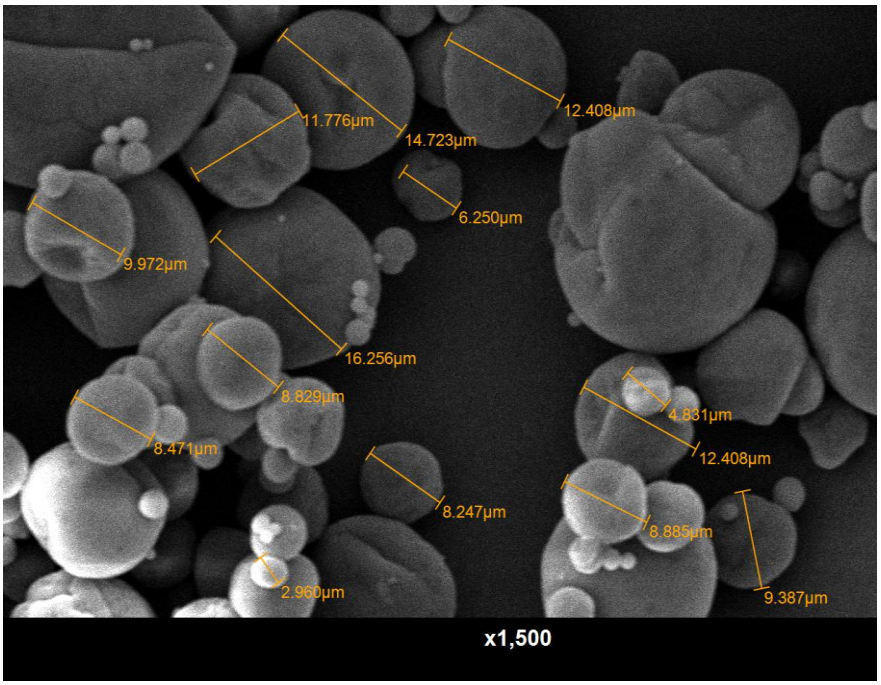
The drying air condition influence is greater compared to emulsion composition. Also atomization controls the particle size of the microcapsules, where nozzle atomization produces larger particle size than wheel atomization. However it is preferable to produce large particles in order to facilitate rehydration where small particles show poor dispersion properties that tend to form lumps on the liquid surface (Assadpoor and others 2008b; Bhandari and others 2007b).

3.3.11 SEM of Powders

Scanning electron microscopy (SEM) of menhaden oil powders MMO, 5MMOBJ and 10MMOBJ, and salmon oil powders MSO, 5MSOBJ, and 10MSOBJ are illustrated on Figure 3.14 and 3.15 respectively.



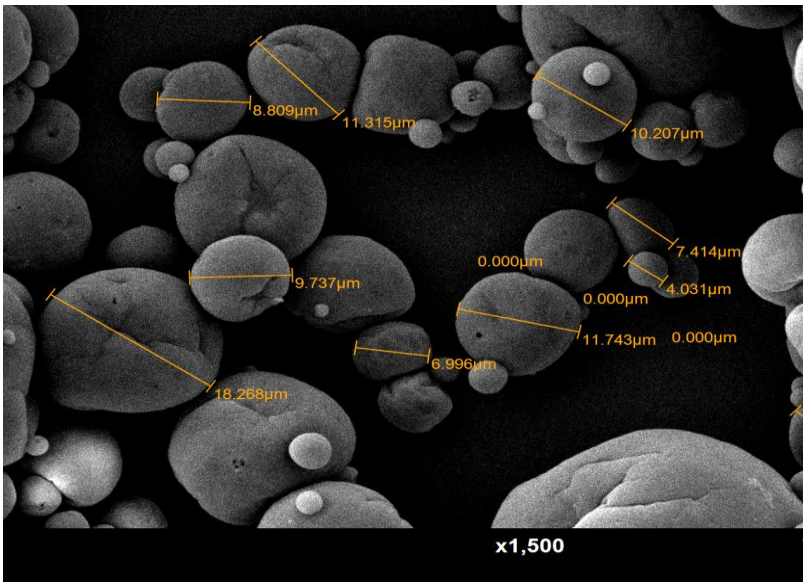
(a)



(b)

Figure 3.14. SEM image of microencapsulated MMO, 5MMOBJ and 10MMOBJ powders (a, b, and c respectively).
MMO= microencapsulated menhaden oil; 5MMOBJ=microencapsulated menhaden oil with 5% blueberry juice; 10MMOBJ = microencapsulated menhaden oil with 10% blueberry juice.

(Figure 3.14 continued)

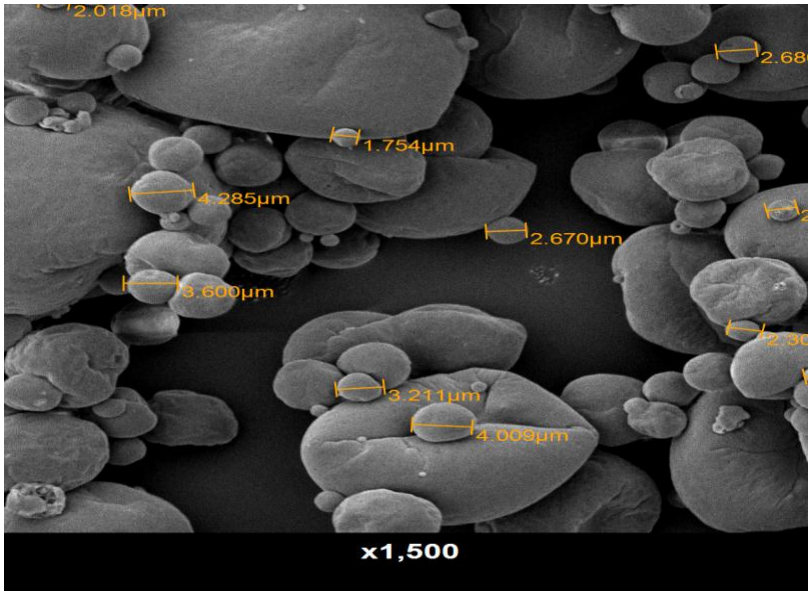


(c)

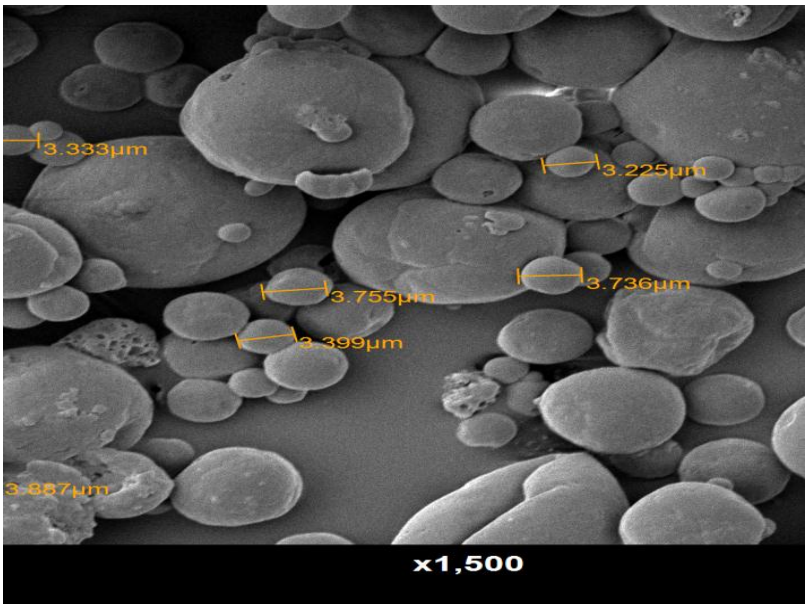
Observation of all menhaden oil powders via SEM (Figure 3.14) showed that there were individual spherical particles with some dents and shrinkage could be attributed to the early stage of drying under high drying rates. This is the typical appearance of a spray drying product. Also there is some aggregation of particles that can be seen in all three powder samples which may be due to surface oil contents. But all three powders do not differ greatly indicating extract addition does not change the morphology of the powders.

There are no distinct cracks on the powder surface observed in all three menhaden powder samples. These powders will provide good protection for the encapsulated oil since there are no cracks on the surface. Cracks are formed during the last stages of drying where expansion of the powder particles may lead to formation of cracks due to weak viscoelastic properties (Bhandari and others 2007a). As mentioned earlier under emulsion rheology, all menhaden powder emulsions have viscoelastic properties which made them able to withstand expansion during the final stages of drying. Surface morphology of the powders mainly related to drying conditions and wall material properties.

Similarly, salmon oil powders shows smooth powders with some degree of shrinkage as menhaden powders. All MSO, 5MSOBJ and 10MSOBJ do not differ greatly in powder structure signifying no extract effect on the powder morphology (Figure 3.15).



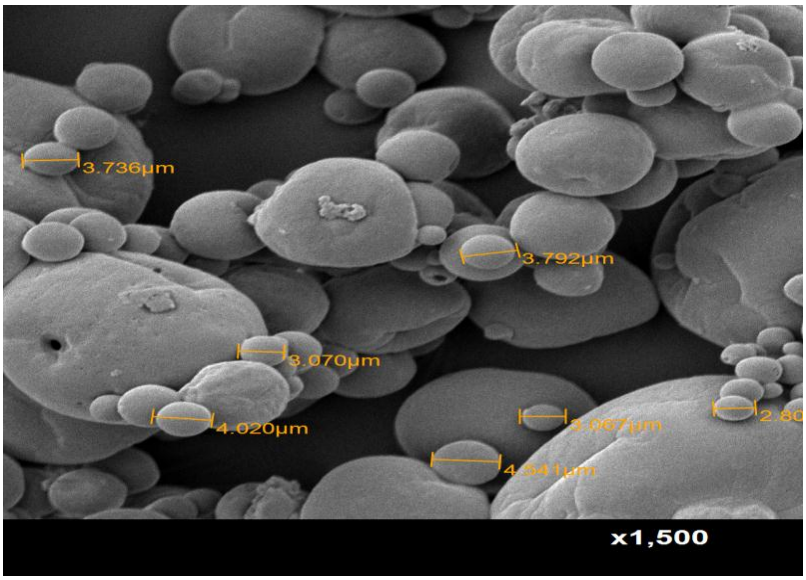
(a)



(b)

Figure 3.15. SEM image of microencapsulated MSO, 5MSOBJ and 10MSOBJ powders (a, b, and c respectively).
MSO= microencapsulated salmon oil; 5MSOBJ=microencapsulated salmon oil with 5% blueberry juice; 10MSOBJ = microencapsulated salmon oil with 10% blueberry juice.

(Figure 3.15 continued)



(c)

But all three powders have broken pieces around powder particles. This could be the impurities present in the salmon oil, retained due to incomplete oil purification. If salmon oil is purified as menhaden oil, these particles will not be present in the powders.

Many previous studies had observed ballooning of particles having wall material as n-OSA starch at high drying temperature of 210/90 ºC (Drusch and others 2007). Ballooning is identified as a phenomenon of droplet instantly covered by a skin rapidly followed by an internal bubble nucleation, and bubbles expand and rupture several times until internal moisture is removed. Finally a smooth particle with a shape of a balloon can be observed (Kolanowski and others 2006; Drusch and others 2009c). But during our experiment we use a temperature of 170 ºC for microencapsulation which showed no result of ballooning. Ballooning temperature is determined by wall material properties and dryer design. Also powders were free from indentation caused by shriveling of particles and deformation during drying at low drying temperatures. Also microcapsules with higher oil loads are known to have less cracks and shrinkage on the surface (Fujishima and others 2003).

3.4 Conclusions

Menhaden and salmon oil emulsions produced with or without blueberry juice had high emulsions stabilities. All of the emulsions showed pseudo plastic (n <1) shear thinning flow behavior, and viscoelastic properties with G`` > G`. The viscous nature was more emphasized than elastic nature due to usage of modified starch as a wall material. The juice addition changed the color of emulsions and microencapsulated powders by increasing a* and reducing L* and b* values. During the emulsification and spray drying process blueberry juice retarded the lipid oxidation. The increased juice addition related to decreased lipid oxidation in emulsions and powders. Also powders with or without blueberry juice did not show significant differences in powder morphology even having a higher oil load above 50%. Particle size of the powders ranged between 20-30 μm. this study indicated that blueberry juice could be effectively added to the fish oil emulsions in order to prevent potential lipid oxidation during emulsification and spray drying process without significantly altering emulsion and powder characteristics.

CHAPTER 4 SUMMARY AND CONCLUSIONS

Blueberries have received greater attention owing to their positive effect on human health and disease prevention. These benefits are mainly related to the presence of polyphenolics, including anthoycanins, anthocyanidins, flavan-3-ol and flavonols in the fruit. The importance of blueberries has greatly emphasized their high antioxidant activity compared to many fruits and vegetables. Recently blueberries are marketed fresh, frozen and processed into a wide variety of products, including fruit juices, purees and concentrates. Blueberry juice processors follow thermal pasteurization in order to comply with the FDA standards of providing 5-log reduction in pertinent micro-organisms present in the juice. But thermal pasteurization is associated with destruction of anthocyanins and sensory properties. Consumers have greater demand for fresh like fruit juices with high contents of bioactive compounds. For this reason non thermal processing techniques are required to test for microbial inactivation and preservation of juice quality. The aim of this study was to compare effects of thermal pasteurization and continuous ultra-sonication processing on physico-chemical and microbiological properties of blueberry juice. Also the aim of this study extended to evaluation of the effect of blueberry extract on lipid oxidation of microencapsulated menhaden and salmon oil.

The specific objectives of the study were to : (1) determine the effect of thermal pasteurization time and temperature on physico-chemical properties and microbial counts of blueberry juice; (2) determine the effect of ultrasonication flow rate and amplitude in a continuous system on physico-chemical properties and microbial counts of blueberry juice; (3) determine the degradation rates of anthocyanins in blueberry juice with thermal pasteurization; (4) prepare blueberry juice and characterize of the juice for anthocyanins, polyphenols and antioxidant activity; (5) prepare and evaluate the physico-chemical, rheological properties, and lipid oxidation of prepared emulsions of menhaden and salmon oil

with or without blueberry juice; (6) evaluate the lipid oxidation and fatty acid methyl ester profile of spray dried microencapsulated powders produced from menhaden and salmon oil emulsions; (7) evaluate microencapsulated powders for powder properties, color and microencapsulation efficiency; (8) estimate the production rate and energy requirement for spray drying of menhaden and salmon oil emulsions in order to produce microencapsulated powders.

The second chapter was devoted to study and compare the effects of thermal pasteurization temperature (80, 85 and 90 ºC) and time (1, 2, 3, 4 and 5 min), and ultrasonication flow rate (24 mL/min and 93.5 mL/min) and amplitude (40%, 80% and 100%) on prepared blueberry juice physical (color), chemical (anthocyanin content, total phenol content, antioxidant activity, pH, ºBrix and titratable acidity) and microbiological (aerobic plate count, total coliforms and yeast & mold counts) properties. Microbial counts of juices reduced with increasing thermal pasteurization and ultrasonication processing conditions. Ultrasonication showed greater reduction in counts with increased amplitude (intensity). The study showed increased degradation of anthocyanins with increased temperature and time in thermal pasteurization while ultrasonication showed no significant difference. The color of the juice changed with thermal pasteurization conditions while all ultrasonication processing conditions yielded no differences. Total phenol content of juice increased with increased conditions of ultrasonication, and in some treatment combinations of thermal pasteurization indicating an extraction of bio-active compounds from suspended juice particles. Antioxidant activity of juices increased with sonication intensity and flow rate related to compound extraction. Antioxidant activity increase associated with thermal pasteurization at higher temperatures and heating time showed a possibility of occurring Maillard browning.

In chapter three, the effect of blueberry juice (BJ) antioxidants on lipid oxidation of microencapsulated menhaden and salmon oil during the time of emulsification and spray drying was studied. All prepared emulsions showed pseudo plastic shear thinning behavior and viscoelastic properties. Although juice addition changed the emulsion pH and color, there was no effect on emulsion stability even at a higher oil load at 57%. The lipid oxidation of fish oil emulsions during emulsification was prevented by blueberry juice addition. The 10% juice added emulsions (10ESOBJ/10EMOBJ) showed no difference during emulsification compared to 5% added emulsions (5ESOBJ/5EMOBJ). 10% blueberry juice added emulsions showed reduced lipid oxidation during the time of spray drying. The juice addition did not show significant difference in produced microcapsules for particle size and microstructure. The particle size of microcapsules ranged from 10-20 μm. The BJ added microcapsules showed better encapsulation efficiencies and lower surface oil contents compared to microcapsules produced without the juice. However BJ changed the color of microcapsules by increasing a* and lowering the L*. There was no significant difference in production rate and energy requirement within salmon and menhaden oil powders.

In summary, ultrasonication continuous system can be effectively used for pasteurization of blueberry juices in order to preserve bioactive compounds and juice initial quality. But further research work is necessary to ensure 5-log reduction. Microencapsulation emulsions can be stabilized using natural antioxidants like blueberry juice in order to prevent lipid oxidation during emulsification, spray drying and subsequent storage. This study demonstrated that ultrasonication has a potential to pasteurize blueberry juice, preserving its initial juice quality. Also blueberry antioxidants can be effectively utilized to prevent lipid oxidation in spray dried fish oil emulsions and microencapsulated powders.

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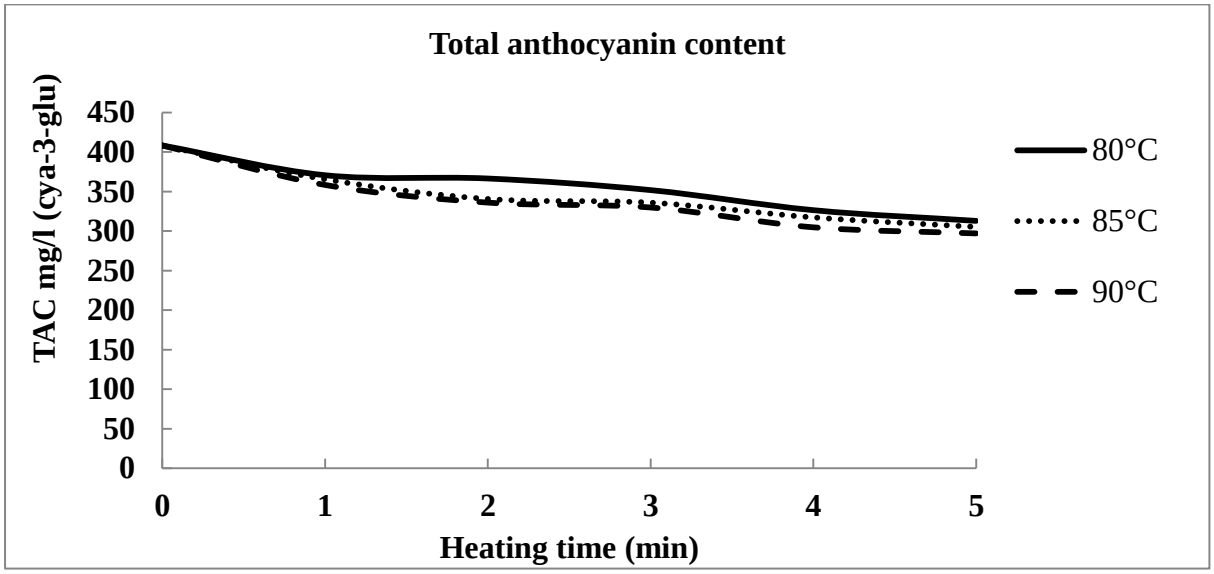
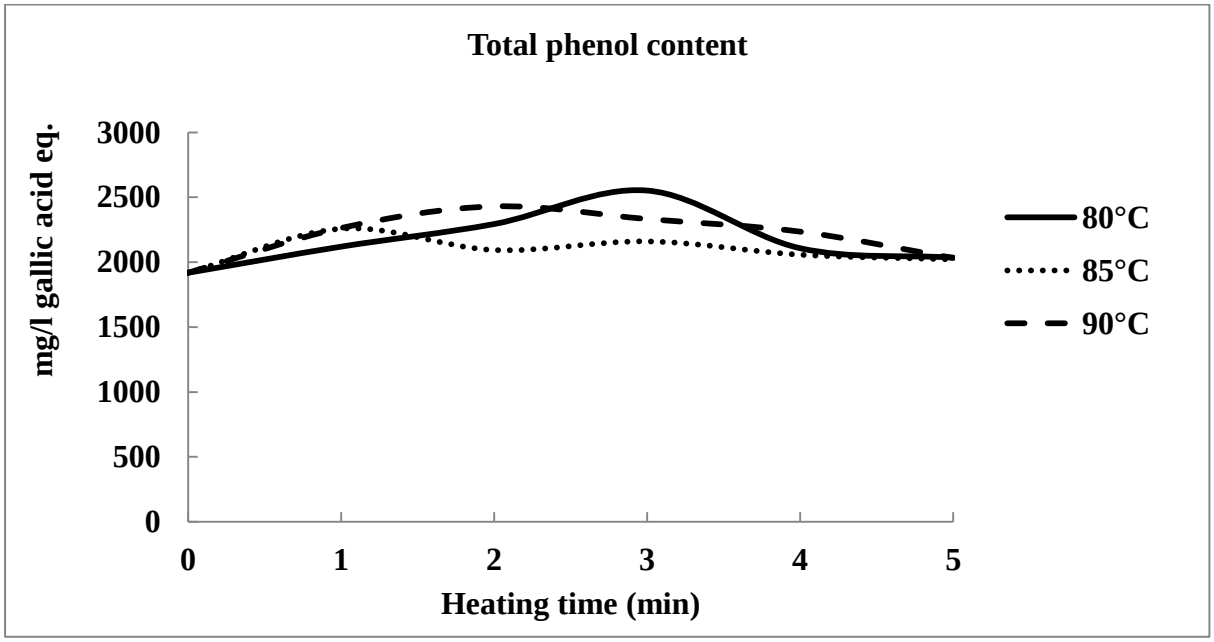
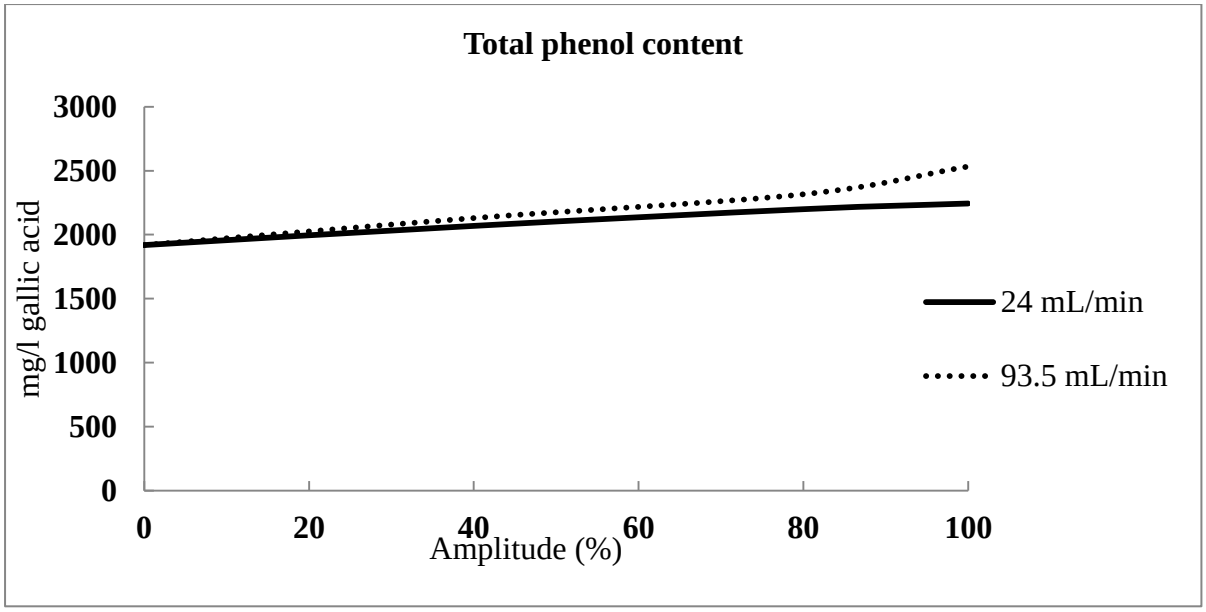
APPENDIX A: BLUEBERRY JUICE TAC, TPC AND AA GRAPHS FOR THERMAL PASTEURIZATION AND ULTRASONICATION



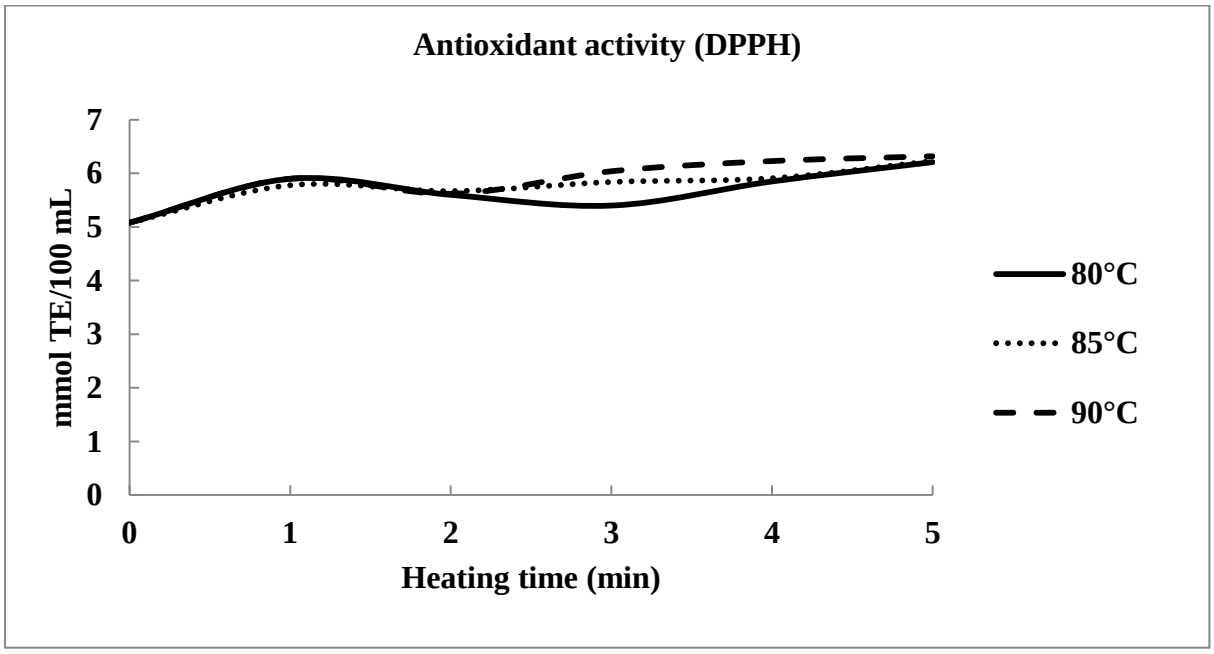
Total anthocyanin content (TAC) of thermally pasteurized blueberry juice as a function of heating time



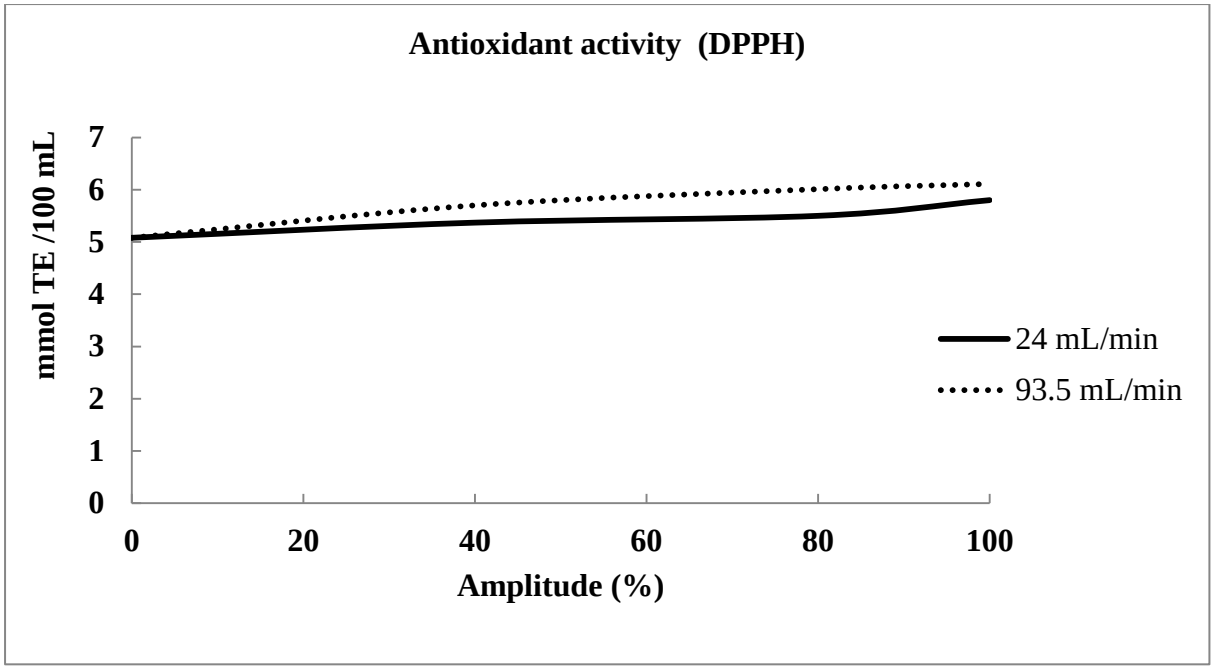
Total phenol content (TPC) of thermally pasteurized blueberry juice as a function heating time



Total phenol content (TPC) of sonicated blueberry juice as a function of amplitude



Antioxidant activity (AA) of thermally pasteurized blueberry juice changes with heating time



Antioxidant activity (AA) of sonicated blueberry juice changes with amplitude

APPENDIX B: SPRAY DRYING PERFORMANCE

During the time of spray drying, temperature, relative humidity, and air velocity of ambient air and exhaust air was measured using an Omega 4-in-1 multifunctional anemometer (Omega Engineering, Stamford, CT, USA). Moisture content of emulsions, microencapsulated powder and dust were analyzed using a SMART system 5 microwave moisture/solids analyzer (CEM Corporation, Matthews, NC, USA). The ambient and exhaust air pipe inner diameters were measured. The temperature of emulsion, powders and dust were measured.

- Actual and estimate powder production rate

The actual microencapsulated powder production rate was calculated according to the procedure given by Bankston and others (2011). The overall mass balance of dry solids (basis of one hour) during spray drying was represented by the Equation (1).

$$me = mP + md \quad (1)$$

Where m_e represents emulsion flow rate (Kg dry solids/h) and m_d represents dust flow rate (kg dry solids /h). Following this equation the estimated microencapsulated powder production rate (mP) was calculated. The mP includes the powder collected from cyclone and powder retained in the spray dryer.

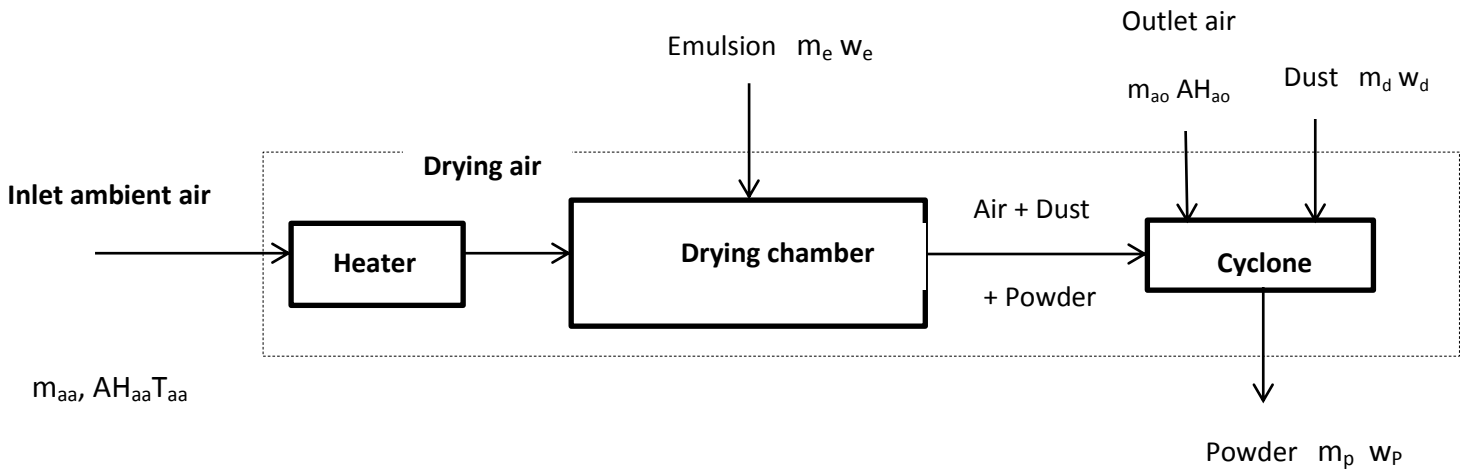
For the purpose of the mass balance calculation, the powder retained in the spray drier and powder collected from cyclone was assumed to have similar physical properties. The actual powder production rate was calculated using collected powder in the cyclone vessel, and it was compared with the estimated production rate (mP).

- Evaporation rate

The total mass balance for water entering and leaving the spray dryer was calculated according to the Equation (2).

$$m_{aa}AH_{aa} + m_e w_e = m_{ao}AHao + m_d w_d + m_p w_p \quad (2)$$

Where m_{aa} and m_{ao} represent flow rate of dry air mass (Kg dry air/h) of inlet ambient air and outlet air respectively. m_e and m_d represent mass flow rate (kg dry solids/hr) of emulsion and dust respectively. AH_{aa} and AH_{ao} represent absolute humidity (kg water/kg dry air) of inlet ambient air and outlet air respectively. The w_e, w_d, w_p represents moisture content in dry basis (kg water/ kg dry solids) of emulsion, dust and product respectively.



Material flow diagram for spray drying of fish oil emulsions

Evaporation rate of the drying process was calculated following two different methods (Bankston and others 2011), as based on moisture uptake by the air (Ev_a), and moisture content of the emulsion, powder collected through cyclone vessel and dust (Ev_p) according to corresponding Equations of (3) and (4).

$$Ev_a = m_{ao}AH_{ao} - m_{aa}AH_{aa} \quad (3)$$

$$Ev_p = m_e w_e - m_d w_d - m_p w_p \quad (4)$$

- Estimation of energy required to produce microcapsules

The Equation (5) was used to calculate the energy required to heat inlet ambient air explained by Singh and Heldman (2001).

$$Q = m_{aa} c_p \Lambda T = m_{aa}(C_{aa} + C_v AH_{aa})(T_{ad} - T_{aa}) \quad (5)$$

Where C_p, C_{aa} and C_v is specific heat (KJ/KgK) of inlet ambient air, inlet ambient dry air and water vapor respectively. ΛT represent temperature difference between inlet ambient air and heated air (K). T_{aa} and T_{ad} represent temperature (K) of inlet drying air and inlet ambient air respectively.

APPENDIX C: SPRAY DRYER PERFORMANCE CALCULATIONS

- Following equation is used to measure dry air mass flow rate (m_{aa} - Kg Dry air / h)

$$maa = \frac{V}{V`}$$

V – Volumetric flow rate of ambient air (m^3/s)

V` - Specific volume of ambient air (m^3/ Kg dry air)

- Following equation is used to measure volumetric flow rate of ambient air (V)

$$V = v \times A$$

v – Average velocity of the ambient air (m/s)

A – Cross sectional area of the inlet air pipe (m^2)

- Following equation is used to calculate specific volume of the ambient air (Singh & Heldman, 2009)

$$V` = (0.082T + 22.4)(\frac{1}{29} + \frac{AH}{18})$$

T – Temperature of the inlet ambient air (°C)

AH – Absolute humidity of inlet ambient air (Kg water/ Kg dry air)

- Absolute humidity of inlet ambient air was calculated according to the procedure described by AIChE equipment testing procedure (2009).

$$AH = 0.622 \times \frac{Pw}{101.325 - Pw}$$

Pw – Partial pressure exerted by water vapor (KPa)

- The partial pressure exerted by water vapor is calculated using following equation (Singh & Heldman, 2009)

$$Pw = Pv \times RH$$

Pv - Saturation pressure of water vapor (KPa)

RH – decimal value of relative humidity

APPENDIX D: SPRAY DRYER PERFORMANCE TABLES

Material and energy balances for a process are important for an industry to control the processing and especially yields of the products. Tables 1 and 2, indicates the material balance for menhaden (MMO, 5MMOBJ and 10MMOBJ) and salmon oil powders (MSO, 5MSOBJ and 10MSOBJ). Also Table 3 and 4 represent the tables for energy balance calculations for menhaden and salmon oil powders. This indicates that increase of spray drying temperature increased the required energy to spray dry. Increase of drying temperature will also increase the cost of production. It should be considered that part of the energy supplied was used for the actual spray drying process, and the rest of the energy was loss with exhaust air and loss from the spray dryer to the outside environment.

Efficient use of energy is important for spray drying activity. According to AIChe equipment testing procedure (2003), optimum condition of spray drying which provides highest thermal efficiency is at the highest temperature difference between inlet and outlet air (ΔT). Thermal efficiency is defined as the ratio of heat used in evaporation to heat input. Increased inlet air temperature increases evaporation capacity of the spray dryer but may affect the product properties. Increase inlet temperatures are known to form hollow particles and also increase thermal degradation. The low inlet temperatures result in low evaporation rates, which produce powders with higher moisture content and agglomerated powders (Bankston and others 2011). Therefore, optimum conditions required to spray dry a specific product needs to be established by trial and error.

Table 1 Mass flow rates and estimated powder production rates of spray drying MMO, 5MMOBJ and 10MMOBJ.

	MMO			5MMOBJ			10MMOBJ		
	emulsion	powder	dust	emulsion	powder	dust	emulsion	powder	dust
MC[a]	68.90±0.11	4.07±0.28	3.20±0.24	64.58±1.10	2.59±0.75	1.98±0.35	66.47±0.87	3.39±1.00	2.63±0.69
MC[b]	2.21±0.01	0.04±0.00	0.03±0.00	1.83±0.09	0.03±0.01	0.02±0.00	1.98±0.08	0.03±0.01	0.03±0.01
$m^a \times 10^2$	82.27±0.14	23.28±0.30	0.875±0.02	76.04±2.01	21.31±0.04	0.88±0.01	78.71±0.11	21.77±0.51	0.77±0.07
$m^b \times 10^2$	25.59±0.11	22.34±0.24	0.85±0.02	26.91±0.15	20.76±0.16	0.86±0.01	26.39±0.71	21.03±0.71	0.75±0.06
$E \times 10^2$		24.74±0.09[a]			26.05±0.15[a]			25.64±0.27[a]	

Values are means and SD of triplicate determinations. [a]Means with same exponents in the each row indicate no significant difference ($p \geq 0.05$).
MMO= microencapsulated menhaden oil; 5MMOBJ=microencapsulated menhaden oil with 5% blueberry juice; 10MMOBJ = microencapsulated menhaden oil with 10% blueberry juice; MC = percent moisture content wet basis and dry basis (kg water/kg dry solids) (a and b respectively); m =mass flow rate wet basis (kg/h) and dry basis(kg dry solids/h) (a and b respectively); E = estimated powder production rate (kg dry solids/h).

Table 2 Mass flow rates and estimated powder production rates of spray drying MSO, 5MSOBJ and 10MSOBJ.

	MSO			5MSOBJ			10MSOBJ		
	emulsion	powder	dust	emulsion	powder	dust	emulsion	powder	dust
MC[a]	69.35±0.26	1.62±0.04	1.2.33±0.12	66.16±0.20	±2.75±0.29	2.17±0.25	66.14±0.06	2.59±0.06	2.13±0.17
MC[b]	2.26±0.03	0.02±0.00	0.01±0.00	1.95±0.02	0.03±0.00	0.02±0.00	1.95±0.01	0.03±0.00	0.02±0.00
$m^a \times 10^2$	82.06±0.49	22.92±0.98	0.78±0.09	81.35±0.46	22.50±0.33	1.18±0.22	79.14±1.71	22.42±0.88	0.61±0.09
$m^b \times 10^2$	25.15±0.36	22.55±0.98	0.77±0.09	27.53±0.23	21.89±0.34	1.15±0.21	27.00±0.60	21.84±0.85	0.60±0.09
$E \times 10^2$		24.38±0.38[a]			26.38±0.01[a]			26.20±0.66[a]	

Values are means and SD of triplicate determinations. [a]Means with same exponents in the each row indicate no significant difference ($p \geq 0.05$).
MSO= microencapsulated salmon oil; 5MSOBJ=microencapsulated salmon oil with 5% blueberry juice; 10MSOBJ = microencapsulated salmon oil with 10% blueberry juice; MC = percent moisture content wet basis and dry basis (kg water/kg dry solids) (a and b respectively); m =mass flow rate wet basis (kg/h) and dry basis(kg dry solids/h) (a and b respectively); E = estimated powder production rate (kg dry solids/h).

Table 3 Summaries of Calculations for the Evaporation Rate and Energy Required to Spray Dry of MMO, 5MMOBJ and 10MMOBJ.

	MMO		5MMOBJ		10MMOBJ	
	ambient	exhaust	ambient	exhaust	ambient	exhaust
Temperature(°C)	24.64±0.83	74.77±0.88	24.35±0.02	72.83±0.85	24.01±0.53	73.33±0.47
Velocity (m/s)	14.55±0.55	3.25±0.02	14.17±0.36	3.23±0.05	15.37±0.95	3.13±0.12
Relative humidity (%)	55.52±3.61	9.30 ±0.12	65.46±3.96	9.43±0.34	66.37±2.21	9.40±0.08
Pipe diameter (m)	0.034	0.075	0.034	0.075	0.034	0.075
Vol. flow rate of air (m^3/h)	47.56±1.81	51.71±0.11	46.32±1.17	51.44±0.75	51.93±3.66	48.29±3.23
Absolute humidity×10^3 (kg water/kg dry air)	10.34±0.68	22.10±0.29	12.23±0.75	22.34±0.80	12.40±0.42	22.26±0.19
Partial pressure (KPa)[a]	1.66±0.11	3.60±0.05	1.95±0.12	3.64±0.13	1.98±0.07	3.63±0.03
Saturation pressure (KPa)[b]	2.98	38.58	2.98	38.58	2.98	38.58
Specific volume (m^3/kg dry air)	0.86±0.00	1.02±0.00	0.86±0.00	1.01±0.00	0.86±0.00	1.01±0.00
Mass flow rate (kg dry air/h)	55.56±5.21	50.75±3.52	54.00±4.42	50.76±3.88	60.58±8.16	47.58±7.13
Specific heat of dry air[c]	1.012	-	1.012	-	1.012	-
Specific heat of water vapor[c]	1.88	-	1.88	-	1.88	-
Evaporation rate (kg water/h)[d]	0.546±0.01[a]		0.474±0.05[a]		0.506±0.08[a]	
Evaporation rate (kg water/h)[e]	0.557±0.01[a]		0.486±0.02[a]		0.516±0.02[a]	
Energy used for spray drying (KJ/kg)	8332.67±389.721[A]		8240.10±308.51[A]		9154..69±607.59[A]	

[a]Partial pressure exerted by water vapor ; [b]saturation pressure exerted by water vapor

[c]specific heat measures taken at ambient air temperature (KJ/Kg K) according to tables of Singh and Heldman (2001).

[d]Calculated based on moisture uptake by the dry air (kg water/h); [e]calculated based on moisture content of emulsion, powder and dust (kg water/h). Values are means and SD of triplicate determinations. [a]Means with same exponents in the each column and [A]means with same letters in each row indicate no significant difference ($p \geq 0.05$). MMO= microencapsulated menhaden oil; 5MMOBJ=microencapsulated menhaden oil with 5% blueberry juice; 10MMOBJ = microencapsulated menhaden oil with 10% blueberry juice.

Table 4 Summaries of Calculations for the Evaporation Rate and Energy Required to Spray Dry of MSO, 5MSOBJ and 10MSOBJ.

	MSO		5MSOBJ		10MSOBJ	
	ambient	exhaust	ambient	exhaust	ambient	exhaust
Temperature (°C)	24.96±0.53	74.17±0.70	25.16±0.47	73.83±0.62	24.76±0.18	74.53±0.45
Velocity (m/s)	13.80±0.08	3.27±0.05	13.57±0.19	3.13±0.12	13.54±0.22	3.15±0.05
Relative humidity (%)	55.61±0.49	9.50±0.16	50.40±0.49	9.73±0.17	56.17±0.29	9.53±0.26
Pipe diameter (m)	0.034	0.075	0.034	0.075	0.034	0.075
Vol. flow rate of air (m^3/h)	45.81±1.07	48.03±3.20	47.33±1.89	46.68±2.57	47.23±1.45	46.93±3.04
Absolute humidity×10^3 (kg water/kg dry air)	10.36±0.09	23.34±0.42	9.37±0.09	23.94±0.43	10.46±0.05	23.43±0.67
Partial pressure (KPa)[a]	1.66±0.01	3.66±0.06	1.50±0.01	3.75±0.07	1.68±0.01	3.68±0.10
Saturation pressure (KPa)[b]	2.98	38.58	2.98	38.58	2.98	38.58
Specific volume (m^3/kg dry air)	0.86±0.00	1.02±0.00	0.86±0.00	1.02±0.00	0.86±0.00	1.02±0.00
Mass flow rate (kg dry air/h)	53.45±4.14	47.14±3.14	55.286.27	45.81±3.57	55.14±6.68	46.00±4.01
Specific heat of dry air[c]	1.012	-	1.012	-	1.012	-
Specific heat of water vapor[c]	1.88	-	1.88	-	1.88	-
Evaporation rate (kg water/h)[d]	0.547±0.10[a]		0.580±0.05[a]		0.500±0.07[a]	
Evaporation rate (kg water/h)[e]	0.565±0.21[a]		0.532±0.04[a]		0.517±0.01[a]	
Energy used for spray drying (KJ/kg)	7996.57±142.59[A]		8244.95±362.56[A]		8262.11±245.89[A]	

[a]Partial pressure exerted by water vapor ; [b]saturation pressure exerted by water vapor

[c]specific heat measures taken at ambient air temperature (KJ/Kg K) according to tables (Table 4.4) of Singh and Heldman (2001).

[d]Calculated based on moisture uptake by the dry air (kg water/h); [e]calculated based on moisture content of emulsion, powder and dust (kg water/h). Values are means and SD of triplicate determinations. [a]Means with same exponents in the each column and [A]means with same letters in each row indicate no significant difference ($p \geq 0.05$). MSO= microencapsulated salmon oil; 5MSOBJ=microencapsulated salmon oil with 5% blueberry juice; 10MSOBJ = microencapsulated salmon oil with 10% blueberry juice.

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Fathima Waheeda Mohideen was born in August 1982 in Colombo, Sri Lanka. She earned her bachelor of agriculture specializing in food science and technology at the University of Peradeniya, Sri Lanka, in March 2008. She joined the Department of Food Science at Louisiana State University for her Master of Science in August 2009. Currently, she is a master's degree candidate and will receive her degree in August 2011.